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STUDIES ON THE GROWTH AND BREEDING OF CERTAIN SEDENTARY ORGANISMS IN THE MADRAS HARBOUR

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Introduction

IN recent years methodical work has been carried out on the growth and breeding of marine animals in order to gain an understanding of life in the sea and considerable information has been gathered on the biology of marine organisms. On the Indian coast, however, excepting for a study of the growth and breeding of the pearl oyster (*Margaritifera vulgaris* Schum.), (Herdman, 1903 and Malpas, 1933) and of the edible backwater oyster

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Ostrea madrasensis Preston* (Hornell, 1910) very little work has been done. Sewell (1925) has recorded his observations on the growth of a few marine forms in the Nicobar Islands. Winckworth (1931) has studied the rate of growth and breeding of *Paphia undulata* (Veneridæ) from specimens dredged in the Madras Harbour and sent to him. Quite recently Srinivasa Rao (1936, 1937, 1938) has studied the habits, rate of growth and bre ding of *Trochus niloticus* Linn. and *Pyrazus palustris* (Linné) in the Andaman Seas with very interesting results. A preliminary survey has also been made by Erlanson in 1936 on the growth and breeding of animals, chiefly boring organisms, in Cochin Harbour.

For purposes of this study the following sedentary organisms that commonly settle down and grow on the concrete piles and other substrata in the Madras Harbour were selected.—(1) *Laomedea* (*Obelia*) *spinulosa* Bale var *minor* Leloup, (2) *Hydroides norvegica* (Gunnerus), (3) *Crisia* sp, (4) *Membranipora* sp, (5) *Ostrea madrasensis* Preston, (6) *Mytilus viridis* L, (7) *Patella* (*Cellana*) *cernica* H Adams, (8) *Balanus amphitrite* Darwin, (9) *Polycarpa* sp and (10) *Diandrocarpa brackenhielmi* Michaelsen.

Environment

This work was carried out in the Madras Harbour (Lat. 13° 06' N, Long. 80° 18' E.) situated in a typical tropical coast. This Harbour (Text-Fig. 1) is an artificial one, with an area of about 200 acres roughly rectangular in shape enclosed by concrete breakwaters. These and the concrete piles offer a good place for attachment to several sedentary organisms. The entrance to the Harbour is in its north-eastern corner and it is here that any roughness in the sea is first felt. In the south-west portion of the Harbour there is the 'Boat Basin' in communication with the main Harbour. This part is farthest away from the entrance and consequently is much less subject to the action of waves. From the Boat Basin a narrow channel leads into a pond of calm sea-water, the 'Timber Pond'.

Jutting into the water from the west Quay is another Quay, the New North Quay, about 600 feet in length. The concrete piles of this afford excellent place of attachment for sedentary organisms and it is in the eastern part of this, where there is considerable wave-action and probably abundant food supply, and where the dirt of the Harbour does not accumulate, that much of this work has been carried out. Projecting into the Harbour from the south wharf are some steel jetties the frame-work of which also serves as substratum for the attachment of these forms.

* *Ostrea virginica* changed into *Ostrea madrasensis* Preston. See Preston, 1916.
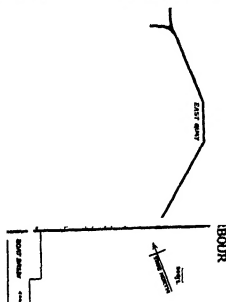
TEXT FIG 1   A sketch of the Madras Harbour showing the places referred to in the text

The temperature of the surface water in the Harbour ranges throughout the year from 22° C to 33° C (Table XIII)   According to the Harbour Engineer there is an average temperature of about 27 6° C. Unfortunately salinity readings were not taken throughout the year but the salinity of the water off the coast of Madras according to Sewell's charts (1929) varies during the different parts of the year as follows:—

September to November	,	30·00 to 32 00 per mille	
December to February		33·25 to 33 50	,, ,,
March to May		33 50 to 33 50	,, ,,
June to August		34 00 to 34·50	,, ,,

The usual tidal range is only from 2 to 3 feet of water   During the rains there is a great admixture of fresh water in the Harbour, especially in those portions farthest from the entrance.

Material and Methods

In studying the rate of growth of organisms suitable objects had to be devised to serve as places of attachment for the sessile organisms   Wooden racks (Pl.-Figs 1 and 2) in which ordinary glass slides 3 inches by 1 inch could easily be inserted were the most useful and the most widely used. This rack was made of two pieces of wood, 4 inches by 2 inches and 1 foot in length, with grooves sawed on one of the broad sides of each piece at a distance of about 1 inch apart. The two wooden pieces were fixed by bolts with their grooved edges opposite each other at a distance of about 2½ inches. Glass slides could be conveniently inserted in these grooves and taken out   To prevent them from being washed off two long but narrow strips of zinc were screwed on to the apparatus at the top and at the bottom, thus keeping the slides in position   By unscrewing one of these strips the glass slides could be easily removed from the wooden rack   The larvae of the organisms studied settled down and grew normally on these slides (Pl.-Fig 3)   At desired intervals the slides were brought to the laboratory in a jar of fresh sea-water and after the early stages were studied under the microscope the slides were conveniently stored in formalin for future reference

Other objects used were (1) wooden pieces scooped out on one side and tied down below low water level and weighted down by short lengths of iron rails, (2) cement blocks suspended in water at definite depths and (3) iron pieces tied down below low water level   The last, however, did not generally allow the larvae to settle down during the first few days because of rusting Because of the large size the wooden and iron pieces and the cement blocks allowed enough space for forms like oysters and mussels to settle and grow on them   Further, definite areas on the concrete walls of the Harbour were scraped down thoroughly and the animals allowed to settle down   These marked areas were frequently examined, the samples removed and measurements taken

In addition, several of the sedentary organisms were collected from boats and buoys. The respective dates on which these were launched or set, after being thoroughly scraped down, were ascertained from the Harbour authorities and from these the approximate age of the organisms collected was calculated   These data were very helpful for verifying the results obtained by the other methods employed.

It was found after some experience that conditions in certain places in the Harbour were more favourable for growth than in others and it is in the former localities that more concentrated work was carried out. Of such places mention may be made of the eastern side of the New North Quay big

buoys Nos. III and IV facing the main entrance to the Harbour and the North and South Buoys in the Boat Basin. Regular observations, week to week, were made from November 1935 to February 1937. Observations of a more general nature, though not weekly, were continued even after the period mentioned. During the period of study visits were made to the Harbour at frequent intervals so that direct information could be obtained of the settling of young ones in various places.

For purposes of growth-study only the best grown individual of each species attached to slides or blocks during a particular period of immersion was selected. To illustrate, from *Hydroides norwegica*, measuring 5 1, 4 7, 3 8, 2 1, and 1 0 mm. in length, obtained from slides kept immersed in water from December 26 to December 30, 1935, the best developed was taken to represent the growth for the four days. After their measurements were taken, the specimens had the condition of their gonads also determined in the living state. Out of a large number of size measurements thus obtained, only those representing the best growth for a particular period have been selected and given in the following tables. It should be remembered that all measurements recorded throughout this paper are actual measurements of the maximum size of individuals and not averages unless otherwise stated and the age in all cases denotes the length of life of the animal after attachment

Rate of Growth, Age at Sexual Maturity and Period of Breeding

Hydrozoa —

Laomedea (Obelia) spinulosa Bale var *minor* Leloup —Of the Hydrozoan colonies getting attached, *Laomedea (Obelia) spinulosa* Bale var *minor* Leloup was chosen. The planula of this form gets attached to and spreads on the surface and from the basal stolon erect individual colonies grow. The maximum height of the individual from the base to the tip is taken to represent the growth. The number of polyps in each stolon is also counted. Leloup (1932) mentions that the individuals of this species do not attain a size of more than one cm. in the tropical seas A maximum length of about 16 mm. is attained here (Table I)

Slides immersed on December 26, 1935, and taken out on January 3, 1936, contained several specimens and some were carrying well-developed gonosomes. Thus, this species attains sexual maturity in 8 days. More often, gonosomes were noticed after 9 or 10 days. The calculation assumes that the larvæ attach themselves to the slides on the day of their immersion. If the attachment takes place actually a day or two after immersion, then the

attainment of sexual maturity must be considered even more rapid. Small Nudibranchs (*Amphorina* ?) were seen browsing on the colonies and to lay their spawn As Orton (1914, 1929) and others record, these Nudibranchs live at the expense of the colonies, rush through their growth-stages and deposit their spawn within a few days Caprellids were found clinging to the colonies mostly during January and February Table I gives the rate of growth of this species

Breeding —A large number of *Laomedea* (*Obelia*) *spinulosa* was found attached from October to February and these carried a considerable number of gonosomes, thus indicating that they breed during this period.

Annelida.—

Hydroides norvegica (Gunnerus) —Of the many sedentary polychætes in the Harbour, *Hydroides norvegica* (Gunnerus) attaches itself in very large numbers, after metamorphosis, to all experimental materials A translucent tube which later becomes calcareous is secreted The tube grows vigorously adhering to the object of attachment but after a fortnight chiefly owing to want of space it grows vertically Soon after, the vertical part of the tube is usually broken off by the action of waves, etc , and it was found impossible to pursue its growth after about two months In the laboratory tanks where artificially fertilized eggs were kept, the worms underwent all the stages of normal development and attained a very large size (105 mm) (Table II)

In recording the growth, not only the maximum length of the tube but also the number of segments of the worm have been taken into consideration In every case the maximum length of the longest tube was accurately measured and then the animal was extracted and the number of segments and the nature of the gonads were made out under the microscope

It was repeatedly found that this worm attained sexual maturity in 9 days after attachment Glass slides let down into water on February 8 and taken out on February 17, 1936, just 9 days later, carried individuals with ripe eggs and sperms Adult worms removed from slides and put in dishes of fresh sea-water, extruded in the case of the male, a milky fluid containing millions of minute sperms and in the case of the female rose coloured eggs. Fertilization took place and gave rise to normal developmental stages

Table II and Text-Fig 2 give an idea of the rate of growth of *Hydroides norvegica* from one day to about two months in the Harbour and to about nine months in the laboratory tanks Text-Fig 3 and Table XI give the growth of the form during all the months of 1936.
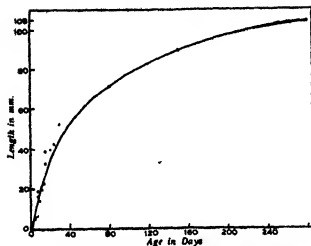
TEXT-FIG 2. Graph showing the rate of growth of *Hydroides norvegica* (Table II)
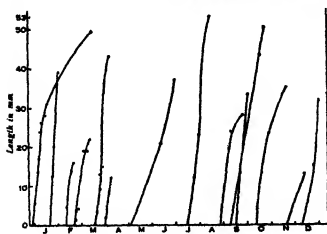
TEXT-FIG 3 Graph showing the growth of *Hydroides norvegica* during all the months of 1936 (Table XI)

Breeding.—It was found that this species was attaching itself to all experimental materials throughout the year Further, artificial fertilization was successful every month of the year. Mature worms, young ones and

larvæ were procured throughout. Breeding, therefore, is throughout the year No special seasonal intensity in breeding was observed.

Polyzoa —

Crisia sp —Among the several species of Polyzoa in the Madras Harbour, a species of *Crisia* is found most frequently attached to the experimental objects This is reddish-brown in colour and grows erect giving rise to many branches The colony starts life as a single disc-like body from the sides of which radical branches are given off in all directions, attaching the selves to the experimental objects Growth is vigorous and in a few days it attains dimensions in which the zooids are difficult to be counted The maximum vertical and horizontal growth of the colony has been measured and taken to represent the size of the colony

When the form attains sexual maturity ovicells arise in or in close connection with the fertile zooecia which give rise to the ovary from which later the embryo is formed. " The ovicell arises in, or is in close connection with, a fertile zooecium which gives rise to the ovum from which the embryos are developed   The number of ovicells of a given colony correspond to the number of fertile zooecia" (Alice Robertson, 1911, pp 226–27) It is the presence of these ovicells that has been taken as the criterion of sexual maturity for this species, since, observations have shown that the zooids are sexually ripe before the ovicells are formed It is an interesting fact that on September 4, 1936, a colony of *Crisia* sp measuring 21 by 14 mm in size, contained more than half a dozen ovicells This colony was found firmly attached to a slide which was immersed in water in the Boat Basin on August 25, 1936 It will thus be seen, that this species could attain maturity in 10 days after attachment though several others have been met with, some of which are mentioned in Table III, where sexual maturity is attained in 16, 19, 20, 24, 25 or more days

Breeding —Young ones and ripe individuals were met with in the blocks as well as in different parts of the Harbour all the year round. During the period of investigation the majority of them settled down and exhibited very good growth during July, August and September This form has, thus, a continuous breeding period lasting throughout the year, with an intensity in July, August and September

Membranipora sp —This encrusting Polyzoan which occurs spreading on shells and other substrata in the Harbour is met with at times in large numbers both on the slides and on blocks. After settling down they grow vigorously and spread on the glass slides into circular or sub-circular colonies.

It has been noticed that on slides of one or two days' immersion one or two initial zooecia of this colony could be found, which rapidly grow all round resulting in about a thousand zooecia in a fortnight

In a colony taken out and examined after fourteen days of immersion, on February 26, 1937, there were some zooecia which had attained sexual maturity

Two measurements across the colony at right angles to each other have been taken to represent the size of this form   Table IV gives an idea of the rate of growth of this species

The breeding of this form was not observed.

Mollusca —

Ostrea madrasensis Preston —Though this is primarily a backwater form occurring in almost all the backwaters of South India, it is also found in fairly representative numbers in the Madras Harbour occurring attached to various objects   The sexes in *Ostrea madrasensis* are separate and fertilization takes place in the water   After a free swimming existence of about a week (Hornell, 1915 and Moses, 1928) the larvæ settle down on any available substrata fixing themselves by their left valve and grow vigorously   After some days' growth they outgrow the size of the slides and the bigger forms were usually obtained from wooden racks and buoys

Sexual maturity is reached in as short a period as 21 days after attachment (Paul, 1937)   Slides that were in water from August 25 to September 15, 1936, contained a large number of *Ostrea madrasensis* attached to them. The biggest among them (Pl -Fig 5) measured 12 5 by 12 0 mm and its gonad was found to contain ripe eggs (Text-Fig 4) and others carried ripe motile sperms

Measurements have been taken in two directions, one from the hinge across the shell to the opposite end and called here as length, and the other, the breadth, the maximum dimension at right angles to this   Pl -Figs 4 to 9 and Table V give an idea of the growth of this form.

Breeding —The periodicity in breeding exhibited by this species is very interesting. During the months of April and May were found a large number of young oysters usually known as spat, attached to the submerged blocks This condition continued and plenty of spat settled down during the following months till the end of October when it ceased   This clearly shows that this form breeds during April to October. However, a study of the animals during the non-reproductive period (November to March) showed that
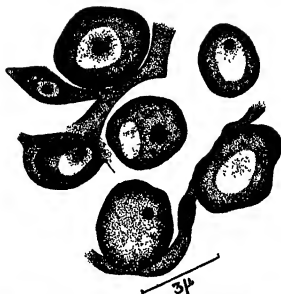
TEXT-FIG 4 Section of the gonad of *Ostrea madrasensis* of 21 days' growth (August 25 to September 15, 1936, size 12 5×12 0 mm) showing ripe eggs along with others in different stages of growth

though breeding as such seems to have stopped, reproduction on a minor scale was taking place, for it was noticed that even during this period they carried well-developed gonads with ripe eggs (with yolk granules distributed throughout the egg) and ripe motile sperms To test the physiological condition of such sexual products the eggs were artificially fertilized in the Laboratory and were found to give rise to early developmental stages Unfortunately further stages in the development were not followed It has to be mentioned that occasionally even during the non-reproductive period there were met with here and there on the slides a few young ones.

It is of interest to compare the results obtained here with those of the same species in the Pulicat back-waters near Madras (Hornell, 1910) with *Ostrea cucullata* along the coast of Bombay (Awati and Rai, 1931) and with *Margaritifera vulgaris* Schum. on the Ceylon coast (Malpas, 1933) In the Pulicat lake it has been found by Hornell that there is a maximum spawning of the oyster in the months of August and September. A second maximal spawning takes place in March and April and " between this time

and August, spawning individuals can always be found " In the Madras Harbour the spawning starts about April or May and continues till it reaches a maximum in September and October, after which it practically stops except for occasional and irregular spawning According to Awati and Rai *Ostrea cucullata* starts spawning along the coast of Bombay in October and continues upto the end of June They could distinguish a *regular* breeding *season* lasting from March to mid-June with intense breeding and an *irregular season* from October to February with a sparse breeding The Ceylon pearl oyster (*Margaritifera vulgaris* Schum), on the other hand, has two spawning maxima (Malpas, 1933), one in July to August coincident with the height of the South-West monsoon and the other in December to January coincident with the North-East monsoon However, I understand from Mr Malpas that during the intervals between the two maxima, irregular spawning takes place

Mytilus viridis L —Though this green mussel forms enormously thick growths on the neighbouring piles it is rarely that the larvae get attached to the experimental materials Slides are not favourite objects for their attachment Occasionally they are met with on the wooden racks, the inner sides of which afford good places for their attachment The sides of freshly scraped buoys offer excellent places of attachment for *Balanus* and other organisms and they in turn offer a foot-hold for the settlement of *Mytilus* larvae Here, owing to the large amount of available space and food, they grow at a rapid rate and attain large size within a short period

After attachment the mussel grows rapidly and 48 days later the form carries ripe eggs (Pl.-Fig 16) or sperms A mussel found attached to a wooden rack that was immersed on September 6, and removed on October 23, 1936, had in its mantle a large number of ripe eggs At this age it had reached a size of 15 5 mm in length and 9 4 mm in breadth. When 93 days old (Pl-Fig 11) a female mussel was observed spawning Table VI gives the rate of growth of this species Pl-Figs 10 to 15 also indicate its growth The mussels have been measured along their maximum length and breadth correct to half a mm. by means of vernier calipers. The details of the breeding of this form will form part of the subject-matter of a separate paper.[*]

Patella (*Cellana*) *cernica* (H Adams) —The gonad of *Patella* (*Cellana*) *cernica* (H Adams), a form occurring among the breakwaters in the Harbour was studied throughout the year 1936 and it was found that at any time of the

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[*] " Observations on the growth of *Mytilus viridis* L., in the Madras Harbour" (unpublished)

year fully adult females and males with ripe eggs and sperms were available. Further, monthly samples of very small individuals (less than 5 mm. in length) of this species were collected all the year round, which showed that they were breeding throughout the year Whether they had any intensity in breeding during a part of the year or not was not observed.

Crustacea

Balanus amphitrite Darwin —Of all the forms studied *Balanus amphitrite* Darwin, was the one which got attached in very large numbers to the experimental materials covering their surfaces to a great extent Small cement blocks immersed in water for 10 to 12 days at any time of the year developed a dense encrustation of *Balanus amphitrite* with practically no interspace between the individuals A square cm. of the colonised area was noticed with as many as 14 young barnacles.

The nauplius larvæ liberated from mature *Balanus* lead a free-swimming existence passing through the many stages of development and finally settle down at the cypris stage After this they grow vigorously and attain a size of 10 by 9 mm in 14 days Pl -Figs 17 to 27 show the growth of this form

Glass slides immersed on February 19 and taken out on March 6, 1936, contained a number of *Balanus amphitrite*, the biggest of which measured 8 8 by 7 3 mm (Pl -Fig 20) When this was carefully removed from the slide and examined it was found that within the brood cavity there were a number of developing nauplii with their appendages not yet fully formed This definitely shows that sexual maturity is attained in 16 days after attachment Other cases have also been met with, in which the animal, at an age of 16 days after attachment, carries either ripe eggs or developing larvæ Table VII and Text-Fig 5 show the rate at which this barnacle grows Text-Fig 6 and Table XII indicate the growth of this species during the various months of 1936 The length of the base through the rostrum and carina is given as the length of the animal and the dimension of the base at right angles to this as the breadth.

Breeding —The sexual periodicity of this barnacle resembles that of the tubiculous polychæte, *Hydroides norvegica* already described A bi-weekly examination of the nature of the gonad of individuals attached to experimental objects and those collected from the Harbour revealed that ripe eggs and developing nauplii were met with in the mantle cavity throughout the year. Though their breeding was followed for more than a year, there does not appear to be any special intensity in the spawning of this form.
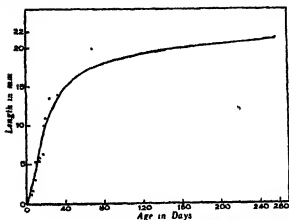
TEXT-FIG 5 Graph showing the rate of growth of *Balanus amphitrite* (Table VII)
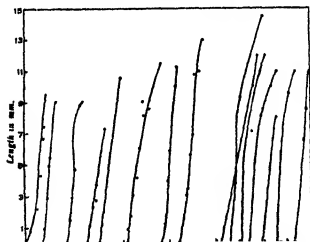
TEXT-FIG. 6. Graph showing the growth of *Balanus amphitrite* during all the months of 1936 (Table XII)

According to Menon (1931) there seems to be in the Madras plankton a maximum number of Cirriped nauplii during April but in the absence of any specific identity of the larvæ, the information does not give any help.

Ascidiacea —

Polycarpa sp.—The simple ascidian was selected for study. This is usually found in groups containing individuals of different sizes closely attached with one another though with no organic connection between them Attached on these are also a large number of Polychætes and Polyzoans The tadpole larve after a free swimming existence attaches itself to the substratum and after metamorphosis grows rapidly

This is a hermaphrite form and the gonad is well developed in 17 days after attachment (Pl -Fig 28) and ripe eggs are met with in 26 days Thus it was found that on a slide immersed on October 2, and taken out on October 28, 1936, the ascidian had reached a size of 22 0 mm in length and 14 0 mm in breadth and contained a well-developed gonad with ripe eggs However, when the gonads of specimens measuring 10 by 6 mm. were examined they were found to contain ripe eggs and these forms would have, in all probability, reached this size in less than 26 days It has to be mentioned, that this species get attached only in certain months of the year and even then not in anything like large numbers Pl -Figs. 28 to 32 give an idea of the growth of this form The distance from the basal portion to the very tip of the branchial siphon and the maximum side to side dimension have been taken to represent the length and breadth respectively, of the form Table VIII gives the rate of growth of this monascidian

Breeding —Sufficient material was not available for a complete study of the breeding of this species but what has been observed is interesting and is set below From the beginning of the year till May none of these individuals were found on the experimental objects but towards the end of May or the beginning of June there was a rich settlement of tiny specimens This continued till the end of October after which practically no individuals were met with Examination of the gonad of this species during the non-breeding period would have been of interest but in spite of repeated efforts the specimens could not be procured from the Harbour But from a study of the organisms settling down on the blocks, it can be said that this species breeds only during the months of May to October, after which period, if at all any young ones are produced, it could only be due to irregular spawning. This form then, seems to have a definite breeding period not lasting the whole year round

Diandrocarpa brackenhielmi Michaelsen —The growth of the compound ascidian, *Diandrocarpa brackenhielmi* Michaelsen, is interesting when compared with that of the simple ascidian. This encrusting colony contains ascidiozooids not grouped in systems and is often met with in extensiv

ANIMAL	J	F	M	A	M	J	J	A	S	O	N	D
LAOMEDEA.												
HYDROIDES.												
MARPHYSA.												
PLATYNEREIS.												
CRISIA.												
OSTREA.												
MYTILUS.												
BALANUS.												
CLIBANARIUS.												
SALMACIS.												
POLYCARPA.												
DIANDROCARPA.												
ACESTROCORUS.												
CLUPEIDS.												
THERAPON.												
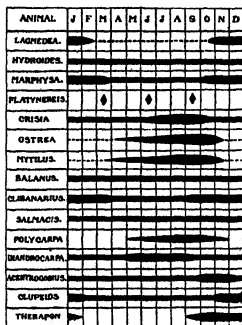
TEXT-FIG 7 Diagram indicating the breeding periods of species studied in this Laboratory Lines of uniform thickness show continuous breeding without any intensity Where thickenings are shown they imply increased breeding activity An interrupted line indicates occasional weak spawning The diamond-shaped thickness shows interrupted periodic spawning

colonies The rate of growth of this is extremely variable and only the maximum actual measurements of the largest individuals of the specified age were taken and are given in Table IX, and hence they represent the most rapid growth obtained during the years of study After the settling down of the tadpole a single individual, the gonozooid (clearly seen on the glass slides), develops from it and later on others arise by repeated budding.

Sexual maturity is attained in 28 days after attachment My efforts to find the nature of the gonad in the living condition and try artificial fertilization were a failure and hence forms representing different ages were fixed in Bouin's fluid, sectioned and studied after staining in iron-hæmatoxylin Forms which were found attached to slides removed on March 2, 1937, after

an immersion for 28 days contained ripe eggs (Pl -Fig 33) along with others in different stages of growth

In 15 days this form reaches a size of 34 by 25 mm. and contains about 110 zooids It was noticed that this ascidian grows to very large dimensions on the wooden racks but such forms have not been taken into consideration for they generally represent the accumulated growth of a number of colonies settled down side by side The maximum size of an individual colony has been measured and the number of zooids counted and are shown in Table IX.

Breeding —The sexual periodicity of *Diandrocarpa brackenhielmi* differs from that of the simple ascidian mentioned already All experimental materials set down at various parts of the year when taken out after a few days invariably carried a few individuals of this form and sexually ripe forms were met with throughout the year Yet, it has been found that during the summer months they occur on the objects in much larger numbers than in the other months of the year For instance, on objects set and taken in the months of November, December and January these colonies were not very numerous Here is again, an instance of a form which though breeding throughout the year has its intensity in breeding during certain months only

Discussion

Rate of Growth.

A study of the tables shows clearly the exceedingly rapid rate of growth of these organisms A comparison of the rates of growth of these forms with those of allied ones in other parts of the world gives confirmatory evidence. *Hydroides hexagonis* grows to a length of 7 0 mm in 16 days and takes 49 days to reach a length of 45 0 mm. at Woods Hole (Grave, 1933), where, according to Fish,[*] the temperature of the sea-water ranges from — 1 to 21 6° C. whereas in the Madras Harbour (temperature ranges from about 22° to 33° C.) *Hydroides norvegica* grows to a length of 39 0 mm in 16 days and attains a length of 43 0 mm in 23 days *Balanus eberneus* grows at Wood Hole to 2·0 by 1 7, 10·0 by 9 0 and 17 0 by 13 0 mm. in 9, 32 and 67 days respectively but its allied species *B amphitrite* grows here to a size of 5 2 by 4 6, 14 0 by 13 0 and 20 0 by 20 0 mm respectively in the three periods mentioned Annandale (1906) has observed in the latter species a growth of 8 0 mm in 22 days (April 17 to May 9) in the Gulf of Mannar A comparison of the growth of *B amphitrite* with that of *B balanoides* in

different parts of the coast of Europe (according to Orton, 1920, the sea temperature off Plymouth ranges approximately from 7° to 16°C)—Herdla, Port Erin and St. Malo—reaching an average length of 6 5, 5 3 and 2 5 to 3 0 mm.* respectively, at the end of one year, also shows the very rapid growth in the Madras Harbour Elmhirst (1923) observed Balanus balanoides (L) reaching a diameter of 13 0 mm in three months in Clyde Sea-area Again, according to Coe (1932), in La Jolla, California (temperature ranging from 14° to 21°C.) Balanus tintinnabulum californicus grows to a maximum length of 20 to 25 mm within a minimum period of 22 weeks The same species has been found to grow to a diameter of 16 0 mm at the end of 8 weeks (Coe and Allen, 1937) In the case of *B amphitrite*, approximately the maximum size (20 0 by 20 0 mm, as recorded in Table VII) is reached in about 68 days

With regard to *Ostrea*, however, as mentioned by Hornell (1910), though the growth is more rapid here than on the European and the North American Coasts, in such places as in the warmer waters of North Carolina the rapidity of growth is almost similar A comparison of the growth of *Mytilus*, in the different parts of the world, will be given in a subsequent paper by the author

Coe (1932, p 41) finds that in La Jolla, California, immersion of experimental materials for at least two weeks is desirable in order to allow the young organisms to grow large enough to be scraped off for later study Erlanson (1936) working in Cochin Harbour says, " Growth was found to be so slow that it was unprofitable to examine the blocks more than once in two months " It is noticed that the Cochin backwaters are very poor in animal life—in species—very probably due to the alternation of almost complete marine and fresh-water conditions It is very natural, therefore, that only very hardy forms (euryhaline) could survive. Other animals which get settled down during the salt-water or fresh-water season will perish when the next season supervenes.

Reference to Text-Figs 3 and 6 (Tables XI and XII), giving in a graphical way the rate of growth during 1936 of *Hydroides norvegica* and *Balanus amphitrite*, respectively, two forms found to breed continuously throughout the year, shows clearly that regular growth takes place during all the months of the year and there is no appreciable retardation of growth during any particular period Here is no adverse season like a very cold winter as in temperate climates to retard growth-rate H S Rao (1937) working in the

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* These figures represent only the average growth and certainly some individuals grow to bigger sizes at the end of one year

Andaman Islands records that in *Trochus niloticus* Linn., there is no slackening of growth during any season of the year, and no definite period of aestivation, though Moorhouse (1932) studying the same species on the Queens, land coast, speaks of a definite slackening of shell-growth during the colder months of the year. Continuous growth is also indicated by Delsman (1929) as a result of his study on the fishes of the Java Sea. From a study of the plankton in the Great Barrier Reef lagoon, it has been found that there is sufficient planktonic food available throughout all the seasons of the year. Marshall (1933) concludes that "the number of diatoms remains comparatively constant throughout the year, there are no great increases such as occurring in temperate waters. There is no seasonal cycle of diatom growth". Russell and Colman (1934) come to almost similar conclusions regarding the zooplankton. It seems, therefore, that there is no difficulty for food for tropical marine animals during the various months of the year.

It is clear from this study that a very large number of animals in the tropics attain sexual maturity at a surprisingly early age. Table X gives a comparison of the age at which related species attain sexual maturity in different parts of the world. It has to be remembered, however, that it is impossible from the nature of things, to get data regarding the growth of identical species in different parts of the world. So we have to content ourselves with making a comparison of the growth of related forms only. Reference to the table also shows that the size at which the local forms attain sexual maturity is very small when compared with that of others. In a few forms like *Ostrea madrasensis* and *Mytilus viridis*, the smallest size at which sexual maturity is attained (12.5 by 12.0 mm. and 15.5 by 9.4 mm respectively) when compared with fully grown ones, is striking. K. V. Rao (1937) working in this Laboratory on the structure and development of *Stiliger gopalai*, found that this ascoglossan attains sexual maturity and gives off spawn within a fortnight. The rate of growth of this form has also been found to be extremely rapid for under normal conditions when food material is available in abundance, they reach within 15 days their maximum size of 9 to 12 mm.

The constant settling down of generations of organisms even of a single species, production of several offsprings in a year and their rapid growth result in an intense competition for food and space and thousands perish in the struggle. It is interesting to see a wooden rack (Pl.-Fig. 2) which had been in water for about a month overgrown with such thick growth of organisms that there is practically no space in between the two wooden pieces. The barnacles have settled down in three or four rows one over the other, with

the result that most of the organisms at the bottom die off for want of oxygen and food. Compound ascidians also grow to enormous sizes spreading like a mat over the Balani just leaving sufficient space for the barnacles to protrude their food-procuring appendages

This rapid rate of growth and the attainment of sexual maturity at a very early age are doubtless governed by a number of physical, chemical and biological conditions such as temperature, salinity, food, sunlight, hydrogen-ion concentration, oxygen content of the water

It has long been known that temperature exerts a profound influence on animals It is believed that on account of the high temperature the rate of metabolism in tropical marine animals is extremely rapid. A number of experimental evidences, such as those on *Amphioxus lanceolatus* and *Beroe ovata*, quoted by Harvey (1928) clearly show that this increased rate of metabolism is due to increase in temperature Russell (1932) as a result of his investigations on the breeding and growth of *Sagitta elegans* in Plymouth has come to the conclusion that the time taken to reach maturity in colder months was longer than in the warmer ones. Thus individuals born in February took 94 days, those born in July 43 days, whereas those born in September took nearly 165 days to attain maturity Hjort (1912, p 762) has brought forth clear evidence to show " that the growth of fishes has proved to be largely dependent on temperature, and that generally the growth of cod-species may be said to decrease, and the age at first maturity to increase the farther north we go " When the rates of growth of different species of the oyster in different parts of the world are compared (Hornell, 1910) those growing under high temperature conditions are found to grow faster Comparing the conditions obtained in the Great Barrier Reef lagoon with those of the English Channel, Orr (1933, p 65) says, " the difference in average temperature between the two areas is large (14° C) and must result in a very much higher rate of metabolism in the Barrier Reef lagoon "

It is generally believed (Aiyar, 1933, p 242) that as a result of the high metabolic activities in the tropics there is a consequent rapid rate of growth and earlier attainment of maturity It has been found to be so with regard to most of the sedentary animals of the Madras Harbour. Delsman, working on the fish eggs and larvae of the Java Sea, has shown that development takes place at a very rapid rate He says (1929, p 2), " With many kinds of pelagic eggs development proves to take no more than one day " " The high temperature of the water (28–29° C on an average) accelerates the hatching of the eggs and the development of the larvae." He cites the example of *Caranx macrosoma*, in which the eggs are produced at about 10 or 11 p.m.,

and the young larvæ hatch at 9 o'clock the next morning Working on the same problem on the Madras Coast, John[*] has obtained very similar results This rapid development has also been shown by Aiyar as a result of his studies on the development of *Acentrogobius neilli* (*Gobius neilli* Day) (1935 *a*) and *Salmacis bicolor* Agassiz (1935 *b*) in Madras As a result of this rapid metabolic activity the duration of life of a large number of the organisms is comparatively shorter than that of similar forms in other places There is no doubt that most of the forms studied pass through several generations in an year

Salinity has also got an important part to play on the growth of these animals Brandt (1897) as quoted by Field (1922), noted that in Kielwight the mussel *Mytilus edulis* grows to a length of 4½ inches, while in the Gulf of Othnia, where the salinity of the water is less, the mussel attains only about half the size of those growing in the saltier regions In Cochin Harbour, where the salinity of the water is reduced by flood waters, it has been noticed (Erlanson, 1936) that growth is very slow Subramaniam and Aiyar (1936) have observed that variation in salinity has got a possible effect on the variation in the size of the eggs of *Dasychone cingulata*, *Salmacis bicolor* and *Clibanarius olivaceus* occurring in Madras. Hopkins (1931) has noticed that the increase in salinity helps the development of oyster (*Ostrea virginica*) larvæ It has also been shown by the same author (1936) that changes in salinity influence the feeding mechanism of the oyster *Ostrea gigas*, and consequently the amount of food taken in and the growth of the species

That abundance of food plays a prominent part on the growth of marine organisms has been accepted by a number of workers (Moore, 1905, Kellogg, 1910, Field, 1922, Orton, 1928 *a*) Field discussing the different factors on which the growth of the mussel, *Mytilus edulis* L, depends, says that the chief one is abundance of food " If food is scarce, growth is retarded regardless of all other conditions " Kellogg has mentioned that especially in plankton feeding organisms the rapidity of currents with the consequent abundance of food results in accelerated growth and perhaps earlier attainment of sexual maturity. It has been observed that in those places in the Harbour, where wave action is negligible, and consequently planktonic food limited—for instance, the Timber pond (Text-Fig 1),—the rate of growth is comparatively less

That sunlight has a marked effect on marine animals is well known It has been found that in the temperate waters the great burst of diatom life

in the early winter is almost entirely due to the availability of solar energy Allen (1907) has shown that there is a direct correlation between the amount of sunlight available in February and March in the British Seas and the mackerel catches in May From a study of the plankton of the Great Barrier Reef lagoon, Russell and Colman (1934) show that the ratio between the minimum and the maximum of zooplankton available throughout the year is not so great there as in the Northern waters or in the Massachusetts Bay and that this is in no little measure due to the fact, that the duration of daylight at different times of the year is approximately constant there when compared with other latitudes.

Breeding —

The question of periodicity in breeding of tropical marine animals is receiving considerable attention at the present day It has been mentioned by Semper (1881) that in the tropics, where the temperature variation is comparatively negligible, there is apparently no periodicity in the breeding of marine animals, chiefly Invertebrates He says (1881, p 135), " During my stay in the Philippines, nothing struck me more peculiar than the evident lack of periodicity in the life of animals, peculiar even to the insects, land molluscs and terrestrial animals I could always find eggs, larvæ and adult individuals of a species at the same time during winter as well as in summer" Orton (1920) who showed that breeding in marine animals is correlated with temperature says that in those parts of the sea, where temperature conditions are constant, or nearly constant and where biological conditions do not vary much marine animals breed continuously However, he suggests that a thorough investigation of this problem is desirable Mortensen (1921) studying the development of tropical Echinoderms, concludes that in the tropics, though the temperature has an important bearing on the rapid development of the larvæ, it need not necessarily result in the continuous breeding of marine animals He says (1921, p 246), " I never found the opportunity for studying the development of *Diadema* until I came to Tobago, B W I. and there found *D antillarum* to have ripe sexual products in the end of March, and when, a week later on, I wanted to start a new larval culture it was impossible to find one specimen containing ripe sexual products, all were empty" He mentions a similar experience with *Echinometra van Brunti, Brissus obesus* and *Stichopus Kefersteinii* In some of the Echinoderms he could detect more than one breeding season in a year He, however, agrees with Orton " that where biological conditions do not vary much marine animals will breed continuously," thereby suggesting that biological conditions may vary in the tropics.

Fox (1924) working in the Red Sea, on the breeding of some Echinoderms, Molluscs and crabs and its correlation with the phases of the moon, infers that breeding is not dependent on temperature Referring to Orton's (1920) statement that the European oyster, wherever it is found, begins to spawn at 15 to 16° C and continues to produce sexual products as long as the temeprature remains above this figure, Fox says, " this is not the case with *Centrechinus*, for its breeding season begins at the Suez some months previous to July at a temperature well below that of July and September, yet from July onwards, with the temperature still rising, the numbers of individuals reaching maturity decline and in September all the breeding ceases although the temperature is still above that at which the breeding season was initiated ". Again the same conclusion is drawn by him for the Alexandria urchins (*Strongylocentrotus lividus*)

From the table giving the occurrence and breeding seasons of ascidians investigated by Berrill (1935), it is seen —(1) that in the Bermudas, where the average temperature is nearly 25° C all the forms studied had regular seasonal breeding, (2) in Plymouth (temperature ranges from 7 to 16° C), though many of the forms observed had regular breeding seasons, others were not seasonal

On the Indian coast, Hornell (1910) working on the backwater oyster, *Ostrea madrasensis* Preston, in the Pulicat Lake (near Madras), comes to the conclusion that breeding is not continuous but takes place at a definite period and is dependent on some factor or factors other than temperature. Discussing the probable cause of breeding he says, " A heightened temperature certainly was not the case, as the temperature of the water *before* the onset of floods was higher than during their continuance " Malpas (1933) records that the breeding of the pearl oyster (*Margaritifera vulgaris* Schum), of the Ceylon coast, during two seasons of the year, does not depend on temperature As a result of a preliminary survey of the growth and breeding of marine animals, especially Teredinidæ, in Cochin Harbour (Erlanson, 1936) it is seen that the forms studied have definite periods of breeding and they do not breed continuously throughout the year.

From what has been recorded by Sewell (1925) with regard to *Littorina scabra* (Linn), *Littorina obesa* Say and *Pyrazus palustris* (Linn) in Nankauri Harbour, Nicobar Islands, and *Mytilus varibilis* Krss. on the coast at Tor in the Gulf of Suez, it is evident that these forms have a particular period of breeding not lasting the whole year round However, much emphasis cannot be laid on these since the breeding period of the forms were not specially investigated. In the case of *Littorina scabra* (Linn.) alone he suggests

that it has two breeding seasons each year, one just before, and another just after the South-West monsoon

Working in this Laboratory on the development of *Salmacis bicolor* Agassiz, a tropical Echinoid, Aiyar (1935 *b*) could obtain from the Harbour ripe specimens and successfully fertilize them artificially in all the months of the year and has thus shown that the form breeds continuously throughout the year. The same author (1933 *a* p 288), found with reference to the breeding of the Polychæte worm, *Marphysa gravelyi* Southern, that " though mature worms are not rare all the year round, there is a distinct reproductive period immediately after the onset of the rainy weather (October) when the egg masses are produced in very great abundance " and that " November to March are crowded months " with regard to the occurrence of Polychæte larvæ in the plankton of the Madras coast (1933 *b*, p 2). He also observed (1935 *a*) *Acentrogobius neilli* (*Gobius neilli* Day) breeding throughout the year in the Adyar backwaters, Madras, but with an intense breeding period during the monsoon in October and November. An examination of the gonad of the brackish-water hermit-crab, *Clibanarius olivaceus* (Henderson) showed (Subramaniam, 1935, p 14) "that they were breeding throughout the year, though the breeding was observed to be particularly well marked during the months of September and March " In his study on the fish eggs and larvæ of the Madras coast, John* found that though the eggs and larvæ of Clupeid fishes are met with throughout the year, there is a definite intensity in breeding from October to January. Studying the growth of *Therapon jarbua*, Ranga Rao‡ found that this fish spawns with the onset of the monsoon in September or beginning of October and continues spawning all through the rainy season, viz, October, November and December, and stops breeding in January. He could notice an intensity in breeding in October when about half of the fish caught contained fully ripe gonads and the other half spent gonads. K V Rao (1937) observed that though specimens of *Stiliger gopalai* could be collected from the Adyar and Cooum rivers throughout the year, a large number of individuals and plenty of spawn were available only when the salinity of the water was comparatively low due to the closure of the bar at the river mouth. Menon (1931, p 491) as a result of his study of the plankton of this coast comes to the conclusion that "most of the organisms exhibit a regular seasonal abundance, and corresponding periods of maxima and minima. This variation, however, is not so clear as in more northern latitudes, as the record of Herdman and other European

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* M A. John, unpublished records

‡ S Ranga Rao, "A statistical study of growth in *Therapon jarbua*" (Unpublished)

workers show " An almost similar conclusion is arrived at by Hornell and Naidu (1924) as a result of their studies on the plankton of the Malabar coast They say (1924, p 151), " Analysis of the foregoing shows that a definite seasonal cycle characterises the maximal abundance of the main classes of organisms of importance in our plankton" It has been recently recorded (Aiyar and Panikkar, 1937) that the Polychæte worm *Platynereis* sp , occurring in the Madras Harbour, exhibits lunar periodicity and has been found swarming on the New-moon day and the day preceding it or (and) following it in the months of March, June and September 1935

In studying the life-history of the Indian Sardine on the Malabar coast, Hornell and Naidu (1924) found that this form spawns from about the end of May to the end of August, with a maximal spawning extending throughout June and July

Anne Stephenson (1934) studying the breeding of marine Invertebrates (Coelenterates, Echinoderms, Molluscs and Crustaceans) in the Low Isles of the Great Barrier Reef observes that breeding takes place in every month of the year, in summer as well as in winter She divides the breeding of animals into four main types —(1) a single breeding period not lasting the whole year round, (2) continuous breeding throughout the year but more active in one part of the year than during the remainder, (3) discontinuous breeding occurring in relation to lunar phases during a longer or shorter part of the year and (4) two spawning periods in the year with a quiescent phase between them

Galtsoff (1934), as a result of his investigations on the oyster, is of opinion that temperature alone is not the paramount cause in the breeding of animals but it depends on many factors which may not directly or indirectly He says, " Statements often found in text-books that under the stenothermic conditions of the tropics breeding appears to be continuous are incorrect" " Even in warmer seas reproduction takes place as regularly as in cold seas "

Whedon (1936) found *Mytilus californianus* Conrad in the region of San Francisco spawning at all times of the year, but with a maximum period of spawning beginning early in October, followed by two other periods of lesser degree in January and February and in May and June He says (1936, p 39), " Temperatures, both of water and of air, doubtless play a part in the rate of development of this spawn, but whether the temperature changes of the region near San Francisco are severe enough to cause spawning, is questionable " Water temperature in the region of San Francisco has varied from approximately 10° C to as high as 17° C over a period of more than a year

H S Rao (1937) discussing this problem of breeding in tropical marine animals puts forth the following questions :—(1) Does the small range of variation in the temperature of tropical seas admit of considering temperature as the main stimulus for breeding ? (2) If the answer to this question is in the affirmative, do the maxima and minima of temperatures attained in various localities act as physiological constants in the breeding of marine animals ? and (3) Do factors other than temperature have any correlation with breeding and if so, to what extent ? He, however, comes to the conclusion that " breeding in tropical marine animals in relation to temperature changes seems to indicate that the evidence in support of the view that the tropical marine animals breed continuously tends to gather weight " Unfortunately he does not refer to the results of the studies on breeding by other workers in India (Hornell, Malpas, Erlanson, *loc cit*) But from his study of the habits and growth of *Pyrazus palustris* (Linne) (Rao, 1938) he thinks that this form breeds in March or April

A review of the literature dealing with the breeding of marine animals clearly shows that though temperature has an important bearing on the breeding of marine animals in the temperate seas, it is not so important a factor bearing on the breeding of tropical marine animals The work of Mortensen, Fox, Hornell, Malpas, Anne Stephenson, Nicholls, Moorehouse, Galtsoff, Berrill and others clearly shows that temperature is not the sole factor influencing the breeding of marine animals in the tropics

A number of workers in the tropics have correlated breeding in marine animals with salinity From a study of the period of breeding of the Ceylon pearl oyster, *Margaritifera vulgaris*, Schum , for many years Malpas (1933, p 21) concludes, " It has been shown in an earlier paper that the Ceylon pearl oyster, *Margaritifera vulgaris*, has two spawning periods each year which reach their maxima in July-August and in December-January, respectively, the former period being coincident with the height of the South-west monsoon when a maximum northerly flow of oceanic water of high salinity enters the Gulf of Mannar and reaches the Pearl Banks at the head of the Gulf, and the latter period being coincident with the height of the North-east monsoon when a maximum southerly flow of water of low salinity, produced by the torrential rains of this monsoon, enters the head of the Gulf from the Palk Strait. This coincidence of the spawning maxima with maximum and minimum conditions of salinity suggests that the oysters are stimulated to maximum spawning by the changes in salinity "

Hornell (1910) made an experimental study to determine the principal factors effecting spawning in the oyster, *Ostrea madrasensis* Preston, in the

Pulicat Lake and summarising the results obtained, says (1910, p. 30), " the maximum sexual activity of the edible oyster of the East coast rivers and backwaters, synchronized with the heavy rains of October and November, October being apparently the maximum   It also furnishes very strong evidence in favour of the view that a rapid reduction in the density of the surrounding water and not an increase of temperature as in European and American waters, is the factor which determines the season at which the majority of oysters shall spawn "

K V Rao (1937) observed that low salinity condition was favourable to the breeding of *Stiliger gopalai*   He found that soon after the opening of the bar at the mouth of the Adyar or Cooum, there was practically no spawn at all and very few individuals could be found   But when the bar was closed and the salinity of the brackish-water was lowered there were plenty of spawn and a large number of young ones, showing thereby, that a sudden fall in the salinity helps breeding and growth of this species.   With regard to *Marphysa gravelyi* Southern, *Clibanarius olivaceus* (Henderson), *Acentrogobius neilli* (*Gobius neilli* Day), *Therapon jarbua* and the Clupeid fishes, it is seen, as already indicated, that breeding or an intensity in breeding takes place soon after the onset of the rainy season, most probably due to the consequent change in salinity

Discussing the occurrence of Siphonophora in the plankton of the Great Barrier Reef lagoon and correlating its comparative absence in it from February to May, with hydrographical conditions existing there, Russell and Colman (1935, p 270) say, " It can at once be seen that the period of absence of most of the species of Siphonophora from the Barrier Reef lagoon coincided with that for low salinity, which was a result of heavy rains prevalent at that time of the year "   In this connection the fact that in the Great Barrier Reef region, the summer months in which " a vast majority of the species investigated was found spawning " (Anne Stephenson) should be the months of maximum rainfall and the consequent lowering of salinity, seems rather interesting

It has been mentioned by Anne Stephenson that the actual breeding season of a particular animal is likely to fluctuate from one district to another   Thus we find that in *Trochus niloticus* L , the spawning period was of at least five months' duration commencing in March in the Low Isles of the Great Barrier Reef (Moorehouse, 1932), whereas the same species was found to breed continuously in the Andaman Islands (Rao, 1937). *Centrechinus* (*Diadema*) *setosus* was found by Fox (1924) to show breeding correlated with the phases of the moon in the Red Sea, whereas the same

species does not show in its breeding any correlation to the lunar phases in the Great Barrier Reef lagoon. It was observed by Moore (1934) that considerable variation occurs in the period of breeding of *Echinus esculentus* at different depths even in localities separated only by a few miles

It is evident, then, that breeding of animals on the Madras Coast is not confined to any particular season of the year   While forms like *Laomedea (Obelia) spinulosa* were most active during the cooler months of the year, the majority of the forms were found spawning throughout the year with an intensity in their breeding during a part of the year   This is shown graphically in Text-Fig 7   The breeding of these can be grouped together under the following types —

(1) Single breeding period not lasting the whole year round, *e g*, *Polycarpa* sp and *Therapon jarbua*

(2) Continuous breeding all the year round but more active during a certain part of it, *e g*, *Laomedea (Obelia) spinulosa*, *Crisia* sp, *Marphysa gravelyi*, *Ostrea madrasensis*, *Mytilus viridis*, *Stiliger gopalai*, *Clibanarius olivaceus*, *Salmacis bicolor*, *Diandrocarpa brackenhielmi*, *Acentrogobius neilli* and the Clupeid fishes, probably also *Patella (Cellana) cernica*

(3) Continuous breeding throughout the year without any special intensity in breeding during any part of it, *e g*, *Balanus amphitrite* and *Hydroides norvegica*   And (4) Discontinuous bre ding related to phases of the moon, *e g*, *Platynereis* sp

Comparing the results obtained here with those of Anne Stephenson (1934) in the Low Isles of the Great Barrier Reef, the breeding seems to be almost similar   Only, forms which breed throughout the year without any intensity during any particular period have not been recorded by her   Species which have two spawning periods in an year have not been so far met with here, but it is not improbable that such forms do occur on the Madras Coast.

From the result of the studies on the breeding of tropical marine animals it is clear, that the balance of evidence favours the idea that temperature is not the all-important factor causing the breeding of tropical marine animals. After discussing the importance of temperature in affecting the breeding of animals in the temperate waters and the occurrence of regular breeding in the animals of a tropical coast like the Great Barrier Reef region (as shown by the results of the Scientific Expedition to the Great Barrier Reef (1928–29), Galtsoff (1934) says, " This fact indicates that the temperature is not always a decisive agent and that there must exist other factors controlling the rhythm of the life processes in the sea".

Summary

1   The rate of growth and period of breeding of the following sedentary organisms in the Madras Harbour have been worked out —*Laomedea (Obelia) spinulosa, Hydroides norvegica, Crisia* sp, *Membranipora* sp. (growth alone), *Ostrea madrasensis, Mytilus viridis, Patella (Cellana) cernica* (breeding alone), *Balanus amphitrite, Polycarpa* sp , and *Diandrocarpa brackenhelmi*

2   The environment and the methods employed in this study are described

3   The rates of growth of the different forms studied, have been compared with those of corresponding or related forms in different parts of the world

4   The species investigated attain their sexual maturity very early in their life

5   There seems to be continuous growth throughout the year

6   Reference is made to the breeding of other tropical marine animals and it is found that breeding takes place —(a) once a year, not lasting the whole year round, (b) twice a year, with a quiescent phase between the two periods, (c) continuously but with an intensity during a certain portion of the year, (d) continuously without any marked intensity and (e) discontinuously depending on the phases of the moon

7   The influence of temperature on breeding in marine animals in the tropics is discussed and it is inferred that temperature is not the only factor influencing breeding

TABLE I   *Rate of growth of Laomedea (Obelia) spinulosa*

Period of growth	Age	Height	No of polyps	Period of growth	Age	Height	No of polyps
	days	mm			days	mm	
Feb 19 to Feb 24, 1936	5	5	5*	Dec 26 to Jan 6, 1936	11	9 0	23‡
Dec 26 to Jan 2, 1936	7	7 5	10*	Jan 22 to Feb 3, 1937	12	10 5	28‡
Jan 22 to Feb 1, 1937	10	5 5	9‡	Oct 12 to Oct 26, 1936	14	16 0	35‡

* No Gonosomes present

‡ Gonosome present

The left-hand column gives the period of growth beginning with the attachment and metamorphosis of the planula   All measurements are of actual colonies and not averages

TABLE II   *Rate of growth of Hydroides norvegica*

Period of growth	Age	Length of tube	No of segments	Period of growth	Age	Length of tube	No of segments
	days	mm			days	mm	
March 15 to March 16, 1936	1	0 9		Jan 18 to Feb 3, 1936	26	39 0	49*
" 28 to " 3	9	1 9		July 13 to Aug, 1918	21	40 0	44*
Dec 26 to Dec 2, 1937	4	5 1		March 15 to April 7, 1936	21	43 0	42*
March 15 to March 21, 1936	6	5 3	18	July 13 to Aug 12, 1936	30	53 0	43*
Dec 26 to Jan 1, 1936	7	5 8		" 13 to " 17, 1936	35		46*
Feb 6 to Feb 16, 1936	9	14 0	30*	Sept. 6 to Oct 16, 1936	40		48*
" 16 to " 1937	9	16 0		May 6 to June 20, 1936	45		48*
Sept 16 to Sept 1, 1936	9	19 0	31*	Sept 6 to Oct 23, 1936	48	42 0	50*
Jan 18 to Jan 3, 1936	11	20 0	43*	Oct 16 to Dec 12, 1936	57		61*
Oct. 12 to " 1936	10	23 0	36*	Sept 16 to Nov 16, 1936	62		56*
Sept 16 to Sept 1, 1936	26	26 5	34*	June 22 to " 18, 1936	149	90 0	51‡
Feb 10 to Feb 1, 1937	14	28 0		June 22 to March 29, 1937	280	105 0	58‡
Sept. 16 to Oct	28	33 0	41*				

* This form attains sexual maturity in 9 days after attachment

‡ Represent the growth of forms in the Laboratory aquarium tanks

TABLE III   *Rate of growth of Crisia sp*

Period of growth	Age	Size	Period of growth	Age	Size
	days	mm		days	mm
Aug. 25 to Sept 4, 1936	10	21 0 × 14 0*	July 13 to Aug. 7, 1936	25	46 0 × 30 0*
July 13 to July 24, 1936	11	21 0 × 14 5	Jan 22 to Feb 17, 1937	27	50 0 × 31 0*
" 13 to " 27, 1936	14	34 0 × 19 5	July 13 to Aug 12, 1936	30	83 0 × 43 0*
" 13 to " 31, 1936	18	50 0 × 32 0	" 13 to " 17, 1936	35	100 0 × 55 0*
June 19 to " 1936	21	35 0 × 35 0	" 13 to " 28, 1936	46	138 0 × 86 0*
July 31 to Aug 21, 1936	21	47 0 × 46 0	Oct 12 to March 30, 1937	169	180 0 × 100 0*
" 31 to Aug 24, 1936	24	63 0 × 35 0			

Size measurements have been taken showing the maximum vertical and horizontal growth of the colony.

* Denotes ripeness of the colony as judged by the presence of ovicells

M. D. Paul

TABLE IV *Rate of growth of Membranipora sp*

Period of growth	Age	Size of colony	No of zooids	Period of growth	Age	Size of colony	No of zooids
	days	mm			days	mm	
July 13 to July 17, 1936	4	3 0×2 5	25	Aug 25 to Sept 7 1936	13	16 0×14 0*	
Jan. 8 to Jan 13, 1937	7	4 0×4 0	30	July 13 to July 27, 1936	14	19 0×19 0*	
July 13 to July 22, 1936	9	15 0×12 5	over 400	,, 13 to ,, 31, 1936	18	31 0×26 0	
Aug. 25 to Sept. 4, 1936	10	14 5×14 0	over 450	,, 13 to Aug 12, 1936	30	41 0×28 2	over 1000
Jan 22 to Feb 3, 1937	12	16 0×12 0		,, 13 to ,, 17, 1936	34	41 0×40 0	over 1000

¹ Ripe zooecia denoting sexual maturity, were obtained

TABLE V *Rate of growth of Ostrea madrasensis*

Period of growth	Age	Length	Breadth	Period of growth	Age	Length	Breadth
	days	mm	mm		days	mm	mm
Aug 25 to Aug 28, 1936	3	0 75	0 75	Aug 25 to Sept 15, 1936	21	12 5	12 0*
Oct. 2 to Oct 12,1936	10	4 4	3 0	,, 25 to ,, 25, 1936	31	15 0	13 0
Aug. 25 to Sept 7, 1936	13	6 3	5 6	May 6 to June 19,1936	44	21 5	14 0*
,, 25 to ,, 10, 1936	16	6 5	6 1	July 13 to Oct 5 1936	84	37 0	34 0*
May 6 to May 25, 1936	19	12 0	12 0	Feb 6 to Oct 7, 1936	243	66 0	71 0*

¹ Sexually mature

TABLE VI *Rate of growth of Mytilus viridis L*

Period of growth	Age	Length	Breadth	Period of growth	Age	Length	Breadth
	days	mm	mm		days	mm.	mm.
July 13 to Aug. 10,1936	28	4 5	9 0	Feb 8 to Aug 10, 1936	184	56 5	30 0
Sept. 6 to Oct 23 1936	48	15 5	9 4*	April 1 to Dec 7, 1936	241	77 5	36 0
July 13 to ,, 1, 1936	84	34 5	19 0	Feb 1 to Oct 7, 1936	243	113 0	52 0‡
March 28 to Sept. 8, 1936	164	52 0	27 0	April 15 to March 2, 1937	321	93 0	39 5
Feb 24 to Aug 10,1936	167	55 5	28 5	May 1 to July 26,1937	445	103 0	44 0

* Sexually mature
‡ Represents exceptionally good growth

TABLE VII   *Rate of growth of Balanus amphitrite*

Period of growth	Age	Length	Breadth	Period of growth	Age	Length	Breadth
	days	mm	mm		days	mm	mm
Jan. 18 to Jan 20, 1936	2	0 9	0 7	Feb 19 to March 6, 1936	16	8 8	7 3*
June 19 to June 23, 1936	4	1 1	0 8	June 19 to July 6, 1936	17	10 0	10 0
May 6 to May 11, 1936	5	1 6	1 4	" 19 to " 8, 1916	19	11 0	11 0
Jan. 18 to Jan 24, 1936	6	2 9	2 8	Jan 31 to Feb 24, 1936	24	13 5	12 0
May 6 to May 14, 1936	8	3 0	2 3	Aug 25 to Sept 26, 1936	32	14 0	13 0
Jan. 18 to Jan 27, 1936	9	4 8	4 0	Sept. 16 to Oct 28, 1936	42	14 5	14 0
Feb 15 to Feb 24, 1937	9	5 2	4 6	July 13 to Aug 23, 1936	46	16 5	13 5
Jan. 18 to Jan 29, 1936	11	5 4	5 0	Aug 25 to Oct 23, 1936	48	20 0	20 0
June 19 to July 1, 1936	12	5 8	5 0	Feb 6 to Oct 7, 1936	243	19 0	19 0
" 24 to " 8, 1936	14	10 0	9 0	Nov 27 to Aug 10, 1936	257	21 5	21 0‡
Jan. 18 to Feb 3, 1936	16	6 2	5 4*				

* Sexually ripe with developing Nauplii larvæ inside
‡ Found attached to a buoy let down into water on February 6 1936
§ Found attached on an oyster shell which was attached to an iron piece ued down on November 27, 1935

TABLE VIII   *Rate of growth of Polycarpa sp*

Period of growth	Age	Length	Breadth	Period of growth	Age	Length	Breadth
	days	mm	mm		days	mm	mm
Oct. 2 to Oct 19, 1936	17	11 0	6 0*	Sept 6 to Oct 23, 1936	47	34 0	15 5‡
" 2 to " 28, 1936	26	22 0	14 0‡	April 1 to Aug 15, 1936	136	51 0	24 0‡‡
" 2 to Nov 2, 1936	30	29 0	12 0‡	" 1 to Sept, 8, 1936	160	63 0	33 0‡‡
July 13 to Aug 28, 1936	45	30 0	16 5‡				

* Gonad of this form well developed
‡ With ripe eggs, sexually mature.
‡ Found attached to the North Buoy, Boat Basin, which was scraped down of all attached organisms, painted and put in the water on April 1, 1936

TABLE IX   *Rate of growth of Diandrocarpa brackenhielmi*

Period of growth	Age	Size of colony	No of individuals	Period of growth	Age	Size of colony	No of individuals
	days						
Feb 19 to Feb 26, 1936	7	7 0 × 4 1	13	May 6 to May 28, 1936	22	62 0 × 38 0	over 300
July 13 to July 22, 1936	9	8 4 × 6 0	15	May 6 to May 29, 1936	23	65 0 × 26 0	over 300
Feb. 19 to Feb 29, 1936	10	8 1 × 7 4	25	July 13 to Aug. 7, 1936	25	65 0 × 33 0	over 300
" 19 to March 2, 1936	12	16 5 × 16 5	34	Feb 2 to March 2, 1937	28	60 0 × 40 0	over 300
" 19 to " 4, 1936	14	27 0 × 23 0	over 100	Sept. 6 to Oct. 16, 1936	40	48 0 × 42 0	over 300
" 2 to Feb 17, 1937	15	34 0 × 25 0	over 100	" 6 to " 23, 1936	47	60 0 × 50 0	over 500
Oct. 2 to Oct. 19, 1936	17	36 0 × 23 5	over 100				
Aug. 26 to Sept 15, 1936	21	46 0 × 28 0	over 100				

Sexual maturity is attained in this species in 28 days.

TABLE X   *A comparison of the age at sexual maturity of certain sedentary forms in different parts of the world*

Species	Locality	Age at maturity	Size at maturity	Author and year
COELENTERATA			mm	
Clytia Johnstoni	Plymouth, England	1 month		Orton, 1914
Tubularia	Cawsand Bay Plymouth	11 days		,, 1929
Companularia flexuosa and C cal ceolifera	Woods Hole Mass	4 weeks		Grave, 1931
Obelia commissuralis		6 ,,		
Laomedea (Obelia) spinulosa	Madras	8 days	5–6	Paul, 1937
ANNELIDA				
Pomatoceros triqueter and Hydroides norwegica	Plymouth	4 months		Orton, 1914
Filograna sp		10½ weeks	54 × 2 7	Grave 1933
Hydroides hexagonis	Woods Hole	59 days	14–19	Paul
Hydroides norwegica	Madras	9 ,,		
POLYZOA				
Bugula flabellata	Plymouth	8 weeks		Orton 1914
	Woods Hole	30 days		Grave
Bugula neritina	La Jolla, California	6 weeks		Coe, 1932
Crisia sp		10 days	21 × 14	Paul
Membranipora sp	Madras	14 ,,	19 × 19	
MOLLUSCA				
Galvina exigua Tergipes	Cawsand Bay, Plymouth	22 days		Orton, 1929
Amphorina	Madras	13 ,,		Paul
Stiliger gopalai		13 ,,		Rao, K V., 1937
Ostrea edulis	English Coast	1 year		Dodd, 1937
Ostrea lurida	La Jolla	3 days	25–3⁴	Orton, 1921
Ostrea madrasensis	Madras	21 days	12 5 × 12	Paul
CRUSTACEA				
Balanus eberneus	Woods Hole	60 days	14 5 × 12 5	Grave ,1933
Balanus tintinnabulum californicus	La Jolla	65 ,,	12–13	Coe, 1932
Balanus balanoides	Plymouth	1 year		Orton, 1914
	Herdla, Bergen	about	6 5	Runnstrom, 1925
,,		1 year	6 5	
	Port Erin	1 ,,	5 3	
Balanus amphitrite	Madras	16 days	8 8	Paul
ASCIDIACEA				
Botryllus violaceus	Plymouth	3 months		Orton, 1914
Leptoclinum (Diplosoma) gelatinosum		3½ weeks		
Botryllus gouldii	Woods Hole	30 days		Grave, 1930
Diandrocarpa brackenhielmi	Madras	18 ,,	60 × 40	Paul, 1937
Molgula manhatensis	Woods Hole	3–4 weeks	70 × 8	Grave, 1933
Ascidia conchilega	Essex Coast	15		Orton, 1914
Polycarpa sp	Madras	16–26 days	22 × 14	Paul, 1937

TABLE XI   *Growth records of* Hydroides norvegica

Period of growth	Age	Length of tube	Period of growth	Age	Length of tube
	days	mm		days	mm
Dec 26 to Dec 30, 1935	4	5 1	May 2 to June 10, 1936	39	20 5
" 26 to Jan 2, 1936	7	5 8	" 2 to " 29, 1936	60	37 0
" 26 to " 5, 1936	10	13 5	June 19 to " 23, 1936	4	2 4
" 26 to " 6, 1936	11	17 0	" 19 to " 28, 1936	9	12 8
" 26 to " 8, 1936	13	24 0	" 19 to July 1, 1936	12	16 0
" 26 to " 10, 1936	15	26 5	" 19 to " 6, 1936	17	25 0
" 26 to " 15, 1936	20	28 5	July 13 to " 17, 1936	4	2 1
" 26 to " 17, 1936	22	31 0	" 13 to " 22, 1936	9	8 5
" 26 to March 14, 1936	79	40 0	" 13 to " 24, 1936	11	17 5
Jan 16 to Jan 27, 1936	9	12 5	" 13 to " 27, 1936	14	17 5
" 16 to " 29, 1936	11	20 0	" 13 to " 31, 1936	18	22 9
" 18 to Feb 3, 1936	16	39 0	" 13 to Aug. 1, 1936	21	40 0
" 8 to " 13, 1936	5	4 5	" 13 to " 12, 1936	30	53 0
Feb 8 to " 17, 1936	9	14 0	Aug. 25 to Sept 4, 1936	13	19 0
" 8 to " 19, 1936	11	16 0	" 25 to " 9, 1936	18	23 5
" 19 to " 21, 1936	2	1 1	" 25 to " 16, 1936	25	30 0
" 19 to " 24, 1936	5	3 8	" 25 to " 23, 1936	30	28 0
" 19 to " 26, 1936	7	4 2	Sept. 6 to Oct 6, 1936	40	43 0
" 19 to March 2, 1936	12	19 0	" 6 to " 23, 1936	48	50 0
" 19 to " 9, 1936	19	19 0	" 16 to " 2, 1936	9	19 0
" 19 to March 16, 1936	26	22 0	" 16 to Oct 16, 1936	16	26 5
March 15 to " 16, 1936	1	0 9	Oct 2 to " 2, 1936	16	33 0
" 15 to " 19, 1936	4	2 9	" 12 to " 26, 1936	14	23 0
" 15 to " 22, 1936	5	5 3	Nov 2 to Nov 18, 1936	36	35 0
" 15 to " 23, 1936	8	9 1	" 20 to Dec. 21, 1936	22	13 0
" 15 to " 24, 1936	9	12 9	Dec 8 to " 18, 1936	10	10 0
" 15 to " 25, 1936	10	15 1	" 8 to " 21, 1936	13	15 0
" 15 to " 27, 1936	12	15 1	" 8 to " 23, 1936	15	15 0
" 15 to April 7, 1936	23	43 0	" 8 to " 28, 1936	20	20 0
" 28 to March 30, 1936	2	1 9	" 8 to " 31, 1936	23	31 0
" 28 to April 7, 1936	10	12 0			

TABLE XII   *Showing the data of growth in* Balanus amphitrite

Period of growth	Age	Length	Period of growth	Age	Length
	days	mm		days	mm
Dec 26 to Jan 2, 1936	7	0 7	June 19 to June 28, 1936	9	3 9
" 26 to " 6, 1936	11	2 2	" 19 to July 1, 1936	12	5 8
" 26 to " 8, 1936	13	2 4	" 19 to " 6, 1936	17	10 0
" 26 to " 10, 1936	15	4 3	" 19 to " 8, 1936	19	11 0
" 26 to " 13, 1936	18	6 7	July 13 to " 22, 1936	9	3 0
" 26 to " 15, 1936	20	7 4	" 13 to " 24, 1936	11	3 4
" 26 to " 17 1936	22	9 5	" 13 to " 27, 1926	14	5 3
Jan 18 to " 20, 1936	2	0 9	" 13 to " 31, 1936	18	6 8
" 18 to " 24, 1936	6	2 9	" 13 to Aug 3, 1936	21	10 8
" 18 to " 27, 1936	9	4 8	" 13 to " 7, 1936	25	11 0
" 18 to " 29, 1936	11	5 4	" 13 to " 12, 1936	30	13 0
" 18 to Feb 3, 1936	16	6 2	Sept. 6 to Oct 16, 1936	40	10 0
" 18 to " 10, 1936	23	9 0	" 6 to " 23, 1936	47	12 0
Feb 19 to " 24, 1936	5	1 3	Sept. 16 to Sept. 23, 1936	9	3 8
" 19 to " 26, 1936	7	2 9	" 16 to " 30, 1936	14	4 5
" 19 to " 29, 1936	10	4 7	" 16 to Oct 28, 1936	42	14 5
" 19 to March 6, 1936	16	8 8	Oct. 14 to " 16, 1936	14	7 0
" 19 to " 9, 1936	19	9 0	" 2 to " 19, 1936	17	9 5
March 15 to " 19, 1936	4	1 0	" 2 to Nov 2, 1936	31	12 0
" 15 to " 23, 1936	8	2 2	" 11 to Oct. 26, 1936	15	8 0
" 15 to " 27, 1936	12	2 7	" 11 to " 28, 1936	17	9 3
" 15 to " 28, 1936	13	3 7	" 11 to Nov 9, 1936	28	10 0
" 15 to April 7, 1936	23	7 2	" 11 to " 16, 1936	35	11 0
April 1 to " 28, 1936	27	10 5	" 2 to " 9, 1936	15	7 5
May 6 to May 8, 1936	2	0 8	" 2 to " 16, 1936	17	9 3
" 6 to " 11, 1936	5	1 6	Nov 16 to Dec 2, 1936	16	8 0
" 6 to " 14, 1936	8	3 0	" 16 to " 10, 1936	24	11 0
" 6 to " 18, 1936	12	4 2	Dec 8 to " 18, 1936	10	4 5
" 6 to " 21, 1936	15	6 0	" 8 to " 21, 1936	13	6 5
" 6 to " 25, 1936	19	9 0	" 8 to " 25, 1936	15	8 3
" 6 to June 19, 1936	44	11 5	" 8 to " 28, 1936	20	11 0
June 19 to " 23, 1936	4	1 1			

TABLE XIII *Mean temperature of air (maximum and minimum) and amount of rainfall at Madras during the period of study*

(Taken from the Monthly Weather Report of the *India Weather Review*, published by authority of the Government of India)

Months	Maximum °F	Minimum °F	Rainfall inches	Surface-water temp * °F
1935				
November	85 5	71 4	6 40	
December	83 4	70 0	2 35	
1936				
January	85 0	68 9	0 10	81 73
February	85 4	73 2	2 53	83 25
March	87 5	72 9	0 90	85 39
April	94 4	78 2	0 46	84 25
May	95 5	81 5	0 02	81 28
June	96 6	80 2	2 82	79 21
July	94 8	79 2	4 87	82 01
August	91 9	77 2	5 99	84 98
September	92 9	77 8	1 93	84 87
October	89 7	76 2	8 16	81 04
November	84 8	73 4	14 53	78 11
December	84 0	71 9	1 94	
1937				
January	83 9	68 6	0 07	79 25
February	86 0	71 9	0	79 33
March	88 9	73 8	0	

* Surface-water temperature of the shore near the Laboratory, an average of readings taken at 9 a m., and 5 p m

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EXPLANATION OF FIGURES

FIG. 1 —Photograph of a wooden rack used in this study The zinc strip and the glass slides inside are seen clearly

FIG. 2 —The same rack, photographed after an immersion for a fortnight in December 1936

FIG 3 —Photograph of 3 glass slides with the attached organisms, immersed in water for (*a*) 13 days, (*b*) 18 days and (*c*) 22 days (4/5 natural size)

FIGS. 4–9—Growth of *Ostrea madrasensis* (FIGS. 4 to 7 ½ natural size, and FIGS. 8 and 9 natural size). FIG. 4. 19 days, 12 0 × 12 0 mm FIG 5 21 days, 12 5 × 12 0 mm. (sexually mature). FIG. 6. 31 days, 15 0 × 13 5 mm. FIG 7 44 days, 21 5 × 14 0 mm FIG 8 84 days, 37 0 × 34 0 mm FIG 9 243 days, 66 0 × 71 0 mm

FIGS 10–15—Growth of *Mytilus viridis* (½ natural size). FIG 10 84 days, 34 5 × 16 0 mm. FIG 11 93 days, 29 0 × 16 4 mm. (observed spawning) FIG 12 135 days, 35 0 × 20 0 mm.   FIG. 13 164 days, 52 0 × 27 0 mm   FIG 14. 184 days, 56 5 × 30 0 mm. FIG 15 243 days, 113 0 × 52 0 mm. (exceptionally good growth)

Fig. 16.—Photomicrograph of a section of the mantle of *Mytilus viridis* 48 days old (15·1 × 9·4 mm.) showing ripe eggs along with different stages in their growth (10 × 40).

Figs. 17–27   Growth of *Balanus amphitrite*. (Slightly bigger than natural size). Fig. 17 9 days, 4·8 × 4·0 mm   Fig. 18. 12 days, 5·8 × 5·0 mm. Fig. 19. 14 days, 10·0 × 9·0 mm. Fig. 20 16 days, 8·8 × 7·3 mm. (sexually mature). Fig. 21 19 days, 11·0 × 11·0 mm. Fig 22. 24 days, 13·5 × 12·0 mm   Fig 23 32 days, 16·0 × 13·0 mm. Fig. 24 43 days, 14·5 × 14·0 mm. Fig 25 68 days, 20·0 × 20·0 mm. Fig. 26 243 days, 19·0 × 19·0 mm. Fig 27 257 days, 21·5 × 21·0 mm

Figs. 28–32—Growth of *Polycarpa* sp (½ natural size) Fig 28 17 days, 12·0 × 6·0 mm. Fig 29 30 days, 29·0 × 12·0 mm. (mature)   Fig 30 45 days, 30·0 × 16·5 mm.   Fig. 31 47 days, 34·0 × 15·5 mm. Fig 32 136 days, 54·0 × 24·0 mm   (*N B* Figures reduced to ½ of magnification given.)

Fig 33 Photomicrograph of a section of the gonad of *Diandrocarpa brackenhielmi* of 28 days' growth showing ripe eggs. (10 × 40)
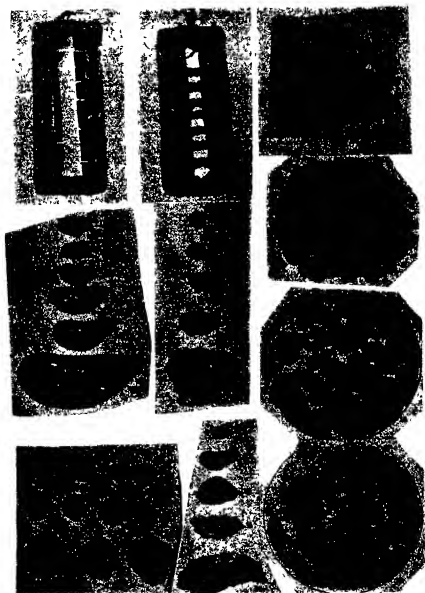
THE EXTERNAL MORPHOLOGY OF THE BRAIN OF *SEMNOPITHECUS ENTELLUS*

(A Comparative Study)

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Introduction

Consequent on a study of the brains of some of the old world monkeys including *Nasalis, Colobus* and *Cynopithecus* with reference to the exhaustive work of Kukenthal and Ziehen on the Primate brain, Beddard (1903) makes the following statement "I cannot distinguish by any tangible differences the arrangement of the furrows in the genera *Macacus, Cercopithecus, Cercocebus* and perhaps *Papio* It appears to me that among the Cercopithecidæ there are only two plans of cerebral conformation, one confined to Cercopithecinæ and the other to Semnopithecinæ" It is proposed in this paper to develop this idea further, by a more detailed study of the external morphology of typical representatives of these two subfamilies. The author's own observations on two brains of *Semnopithecus entellus* form the basis for the comparison. Both these brains were practically alike in their topographical details. The description of the brains of *Macacus* and other monkeys have been taken from studies by Elliot Smith (1902), Beddard (1903), Duckworth (1915), Tilney (1928) and Hines (1933), and in addition a personal examination has been made of the brain of *Macacus sinicus*.

General Features of the Brain of Semnopithecus entellus

Viewed from above the entellus brain has a broad oval outline with the narrow end forwards. The cerebellum is completely hidden by the cerebrum. The frontal pole is narrow, the occipital poles are full and rounded. The orbital surface shows the characteristic keel, medially, and hollow, laterally. The inferior surface of the cerebrum is not as deeply moulded by the cerebellum as in *Macacus*. The broadest part of the brain corresponds to the region behind the upper part of the parallel sulcus. On the medial surface of the sagittal section the relatively large corpus callosum is well made out. The continuity between the septum pellucidum and the gyrus

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subcallosus (parts of the original paraterminal body) is seen (Text-Fig. 2 B) and a flattened band of nervous tissue passes downwards from the lower part of the sep um pellucidum in front of the anterior commissure to become the diagonal band of Broca

Measurements of the Brain of a Young Adult Female Entellus Monkey

Maximum length of brain	6 85 cm
Maximum width of brain	5 95 „
Encephalic index	87
Length of corpus callosum	3 05 cm
Thickness of corpus callosum at splenium	0 4 „
Precallosal length, i e , the distance between the genu and frontal pole	1 6 „
Postcallosal length, i e , the distance between the splenium and occipital pole	2 35 „
Vertical measurement of anterior commissure	0 3 „
Antero-posterior measurement of anterior commissure	0 2 „
Weight of brain, with meninges immediately after removal	114 gm
Weight of brain, without meninges and vessels after prolonged hardening in 5 per cent formalin .	86 5 „
Weight of forebrain	73 5 „
Weight of midbrain	0 75 „
Weight of hindbrain	12 25 „
Proportions of forebrain midbrain hindbrain	85 1 14

Fissuration of the Cerebrum in Entellus

The superolateral surface (Text-Figs 1 A, 2 A)

The central sulcus extends downwards and slightly forwards from a point near the middle of the medial margin and finally makes a terminal bend backwards The length of the central sulcus is 3 0 cm On the surface of the cerebrum in front of the central sulcus three sulci are seen, viz , s rectus, s arcuatus and s precentralis superior The sulcus rectus is nearly parallel to the inferior border near the frontal pole and is 1 9 cm long The sulcus arcuatus is 2 5 cm long and has a course directed from below upwards and forwards The arcuate nature is such that in the lower part it runs in a nearly coronal direction and then terminates anteriorly in a nearly sagittal direction. The superior precentral sulcus is a small one in front of the upper part of the central sulcus

TEXT-FIG 1   Superior (A) and Inferior (B) views of the cerebral hemispheres

The *Sylvian fissure* has a general oblique direction from below upwards
and backwards   Posteriorly its terminal part does not unite with any other
sulcus   On opening the lips of the anterior part of the Sylvian fissure the
insula is seen   The submerged part of the insula is bounded above by the
suprasylvian fissure, whose anterior end extends forwards on the deep aspect

of the operculum, but is not visible on the superficial surface A *pseudo-sylvian fissure* limits the insula below The anterior limiting sulcus of the insula (s fronto orbitalis) is absent and the insula becomes continuous with the posterior part of the orbital surface without any line of demarcation Parallel to Sylvian fissure but more extensive than it inferiorly as well as superiorly is the *superior temporal sulcus* (parallel fissure) It does not join any other sulcus The *inferior temporal sulcus* is represented by three small detached sulci near the lower part of the temporal lobe The middle one is the smallest, being just a dimple

The *intraparietal sulcus* starts midway between the central sulcus and Sylvian fissure and passes at first obliquely upwards and backwards arching over the upper ends of the Sylvian fissure and superior temporal sulcus, the intraparietal then gives off a short medially directed transverse branch in front of the arcus parieto-occipitalis, then turning downwards and backwards the intraparietal joins the lunate sulcus The *sulcus postcentralis superior* is seen as a coronal sulcus parallel to and behind the upper part of the central sulcus In the inferior parietal lobule in the region between the intraparietal sulcus above, the posterior part of the Sylvian fissure below and the superior temporal sulcus behind is a small distinct sulcus of doubtful identity whose significance will be discussed subsequently

The *lunate sulcus* does not touch the superomedial or inferolateral borders It comes close to the superomedial border but is separated by a considerable interval from the inferolateral border The sulcus is concave forwards in its upper part and convex forwards in its lower part The superomedial portion of the lunate sulcus forms the posterior boundary of the arcus parieto-occipitalis The intraparietal sulcus joins the lunate nearly a third of the way down from its upper end The lower portion of the sulcus lunatus and the superior temporal sulcus come close to each other but are distinctly separated by a gyrus on whose surface a short unidentified antero-posterior sulcus is seen The *inferior occipital sulcus* forms a curve concave upwards situated about midway between the lower end of the lunate sulcus and the inferolateral border The posterior end of the inferior occipital sulcus has the appearance of being bifurcated The upper limb of the bifurcation, which is the real continuation of the sulcus, passes on to the lateral surface of the occipital lobe The lower limb of the bifurcation is, however, deeply separated from the rest of the sulcus by a submerged gyrus indicated by two dots in the course of the sulcus in the figures This deeply detached sulcus passes medially towards the inferior surface Its significance will be discussed along with the collateral sulcus In the region situated between the superior temporal sulcus in front, the lunate sulcus behind and the
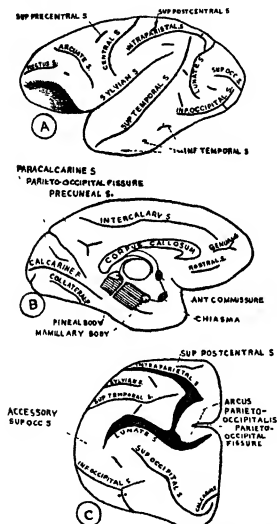
TEXT-FIG 2   Lateral (A), Medial (B) and Posterior (C) views of the left cerebral hemisphere
The posterior view is drawn on a higher magnification and the lips of the lunate sulcus and
intraparietal sulcus are represented slightly opened out   The arcus parieto-occipitalis is
seen fully exposed

inferior occipital sulcus below, is an unidentified sulcus having a coronal direction The significance of this sulcus will also be discussed subsequently The lateral surface near the occipital pole shows the *superior occipital sulcus* and another small sulcus probably accessory to it.

The medial surface (Text-Fig 2 B)

In the circumcalloseal zone on the medial surface the following sulci are seen (1) a *rostral sulcus* situated midway between the rostrum of the corpus callosum and the medial orbital border, (2) a *genual sulcus* in front of the genu of the corpus callosum, and (3) an *intercalary sulcus* starting above the genual sulcus and passing at first backwards parallel to the upper surface of the corpus callosum and finally taking an obliquely upward course towards the superomedial border The termination of the upturned part of the intercalary is situated along the superomedial border about midway between the superior postcentral sulcus and parieto-occipital fissure.

In the region corresponding to the precuneus, there is a triradiate *precuneal sulcus* (compensatory sulcus) placed above the splenium of the corpus callosum It consists of a horizontal element and a vertical element.

The *parieto-occipital fissure* is a deep cleft beginning on the medial surface a little behind the splenium and directed upwards and backwards It does not meet the calcarine fissure inferiorly, being separated from it by a gyrus The upper end of the parieto-occipital fissure cuts the superomedial border and extends on to the lateral surface where it is bounded by the well-marked arcus parieto-occipitalis (Text-Fig 2 C) The arcus is limited in front by a transverse limb of the intraparietal, laterally by the posterior part of the intraparietal and posteriorly by the medial part of the lunate sulcus On opening the lips of the parieto-occipital fissure the fossa shows hidden in its depth a sulcus placed in relation to its floor along the anterior wall and another very small sulcus on its posterior wall Posterior to the parieto-occipital fissure is a small sulcus on the cuneus, probably a *paracalcarine sulcus*

The *calcarine fissure* begins on the medial surface near the occipital pole above the superior occipital sulcus and then runs forwards and downwards on to the inferior surface It is a deep single sulcus and shows no bifurcation posteriorly and no hidden gyrus in its depth.

The inferior surface (Text-Fig 1 B)

The *rhinal fissure* extends backwards from the incisura temporalis and forms a lateral boundary to the pyriform area The *Collateral fissure* begins posteriorly near the occipital pole and runs forwards for nearly two-thirds

of the length of the occipitotemporal part of the inferior surface There is a submerged gyrus in its depth behind its middle showing that it is constituted of two united sulci It also shows two laterally directed rami from the hinder part of the anterior segment Mention has already been made in the description of the inferior occipital sulcus of a small sulcus apparently united to its posterior part but deeply separated from it by a submerged gyrus Between this small sulcus and the collateral fissure is another small detached sulcus These two small sulci together probably represent the *transverse collateral sulcus* Between the collateral sulcus and the anterior end of the calcarine fissure a small sulcus is present This is probably the beginning of a *paracollateral sulcus* The orbital part of the inferior surface shows a gently curved S-shaped sulcus, the *orbital sulcus*, along with small accessory dimples laterally and medially The *sulcus olfactorius* is absent

Comment on Cerebral Fissuration

Inferior parietal lobule —Even without going into a detailed consideration of the areas of cortical localization and cytoarchitectonics, it will be generally admitted that the inferior parietal lobule, i e , the region roughly between the intraparietal sulcus in front and above, and the sulcus lunatus behind, is an association area where sensory impressions of touch, hearing and vision are pooled together The separation of the terminal part of the Sylvian fissure and superior temporal sulcus that occurs in entellus indicates an expansion of this cortical area In addition certain new sulci have appeared in this region (1) A small sulcus has appeared between the intraparietal sulcus and posterior part of the Sylvian fissure It is the superior parallel sulcus (ascending I of Kappers) This identification is in consonance with the interpretation by Beddard (1903) and Shellshear (1927) Beddard also considers that the existence of this sulcus differentiates the brain of *Semnopithecus* from that of *Nasalis* (2) The gyrus that separates the lunate sulcus from the superior temporal sulcus has a small sagittal sulcus on it This is the sulcus prelunatus (3) In the lower part of the triangular area between the superior temporal, lunate and inferior occipital sulci, there is a sulcus in the coronal plane (see Text-Fig 2 A) This is probably the beginning of an anterior occipital sulcus (ascending III of Kappers) The separation of the Sylvian fissure from the superior temporal sulcus and the appearance of these three sulci in the inferior parietal lobule of the *Semnopithecus* for the first time in the evolution of the Primates are evidences of the expanding growth tendencies of this region

The intraparietal sulcus —The transverse branching of the intraparietal in front of the arcus parieto-occipitalis indicates a tendency for the intra-

parietal to become more complex The terminal outward bend of the intra-parietal sulcus prior to its joining the lunate sulcus is also another feature in which *Semnopithecus* differs from *Macacus*

The occipital lobe —The definite decrease in the operculation of the sulcus lunatus and inferior occipital sulcus in *Semnopithecus* as compared with *Macacus*, associated with the constant appearance of a superior occipital sulcus and accessory superior occipital sulcus, shows a backward shift-ing of the striate area by the expanding peristriate cortex The formation of the superior occipital sulcus appears to be a means of accommodating " pushed back " striate cortex in the limited space available Transverse and sagittal sections through the occipital cortex when examined with a hand lens show the stria Gennari extending up to the posterior lip of the lunate sulcus but not extending into the cortex in the depth of the sulcus The stria Gennari is also made out on the sides of and in the depths of the superior occipital sulcus and the posterior part of calcarine fissure

The sulcus cinguli —The sulci that make up the sulcus cinguli of man, e g , the rostral sulcus, genual sulcus and intercalary sulcus are all present in *Semnopithecus entellus* But the terminal upturn of the intercalary is situated midway between the superior postcentral sulcus and parieto-occi-pital fissure and appears to be far too back from the probable posterior limit of the paracentral lobule It is therefore likely that the terminal part of the intercalary does not correspond to the terminal upturn of the sulcus cinguli of man

The precuneus —The precuneus is definitely larger in *Semnopithecus* than in *Macacus* It is this preponderance of the precuneus over the cuneus that gives a posterior inclination to the parieto-occipital fissure in entellus as contrasted with the upward and forward course of the parieto-occipital fissure in *Macacus* In Elliot Smith's diagram of the medial surface of the brain of *Macacus sinicus* (fig 241, R C S Cat , 1902) the precuneal sulcus is absent, and in her diagram of the rhesus brain Hines (1933) shows a small precuneal sulcus, with a query On the contrary Duckworth (1915) clearly indicates a compensatory sulcus (sulcus precunei) in *Nasalis* and it it equally well marked in *Semnopithecus entellus*

The parieto-occipital fossa —The parieto-occipital fissure is really a complex of submerged sulci, and so it is appropriately called a fossa The identification of its constituent elements is based on Elliot Smith's interpret-ation of the parieto-occipital fossa (1902 and 1904) The sulci α, β and γ described and illustrated in fig 241 of R C S Cat (1902) are named incisura parieto-occipitalis, s limitans precunei, and s paracalcarinus, respectively

In the entellus the incisura parieto-occipitalis occurs separated from the intraparietal by a well-formed arcus parieto-occipitalis and the fossa proper is formed by the submergence within it of the incisura parieto-occipitalis and sulcus limitans precunei, due to an overgrowth of the two lips. The sulcus paracalcarinus, however, is not yet submerged and occurs in the cuneus behind the parieto-occipital fissure. Of the two opercula of the fossa, the anterior operculum seems to show greater growth tendencies than the posterior.

The calcarine fissure.—The posterior T-shaped bifurcation of the calcarine fissure usually seen in *Macacus* is absent in *Semnopithecus entellus*. But it has been occasionally noted in *Semnopithecus* (Beddard, 1903, Elliot Smith, 1902) though not so well visible on the dorsal view of the undivided brain as in *Macacus*.

The collateral fissure.—This fissure is longer and better constituted in *Semnopithecus* than in *Macacus*. It gives evidence of being a union of two sulci, by the presence of a submerged gyrus in its depth. It also shows a tendency to develop two laterally directed rami about its middle. The occurrence of transverse collateral and paracollateral sulci, has already been noted. These are pointers to an expansion of the area around the collateral fissure.

Discussion on Cerebral Fissuration of Semnopithecus entellus

From the foregoing comments on the fissuration of the cerebrum, it becomes clear that the fissural pattern of *Semnopithecus entellus* in addition to being different from what occurs in the Cercopithecinæ is also definitely of a more advanced type. Beddard (1903) suggests from the view-point of cerebral morphology that Colobus ' is to be placed with the Cercopithecinæ." The comparison of the brain of *Nasalis* and *Semnopithecus entellus* has already shown that in the formation of the sulcal pattern of the inferior parietal lobule there is a marked advance shown by the entellus monkey. Thus the observations herein noted and the relevant comments made on them lead to the inference that among all the Cercopithecidæ the entellus monkey probably shows the highest cerebral development. In describing the parieto-occipital fissure of *Hylobates hoolock*, Elliot Smith (1902) says " the series of modifications necessary to convert the brain of a *Macacus* into that of a *Semnopithecus* are carried a stage further in the case of *Hylobates*" This intermediate position of the *Semnopithecus* between *Macacus* and *Gibbons* is shown by the entellus not only in the region of the parieto-occipital fossa but also in the whole gamut of its cerebral fissuration. More confirmatory

evidence will be adduced from the cerebellum and brain stem described below

The Cerebellum in Entellus

Nomenclature—The old nomenclature for the parts of the cerebellum has been purposely ignored. Nor is it considered necessary here to go into a discussion of the nomenclatures according to various authors (Bolk, Ingvar, Elliot Smith, Bradley, Abbie, and Larsell) who have worked on the cerebellum. A summary of the cerebellar subdivisions with the notations of the various nomenclatures used is found in pp 788–89 of *The Comparative Anatomy of the Nervous System of Vertebrates* (Kappers, Huber and Crosby, 1936). It indicates the complications present. The writer has conveniently adopted the method of subdivision and naming followed by Ranson (1937) in describing the material on hand.

General features (Text-Figs 3, 4)—The cerebellum of the entellus is 4 3 cm in transverse diameter and presents the characteristic foliated appearance. The dumb-bell shaped form of the human cerebellum is, however, not present. On viewing it from above, the median part shows a smooth elevation with gentle depressions on either side and the lateral parts bulging out beyond. The three pairs of brachia were as usual.

The anterior lobe (Text-Figs 3, 4)—The parts in front of the fissure prima, the deepest fissure in the cerebellum, constitute the anterior lobe. On the cut face of the median sagittal section, the anterior lobe appears to be the largest lobe, and its sectional area calculated from a tracing on graph paper is about 48 per cent of the total sagittal sectional area of the cerebellum. The anterior lobe consists of three subdivisions (1) The *lingula* comprises five small folia situated on the anterior medullary velum and demarcated from the central lobule by the sulcus postlingualis (2) The *central lobule* consists of six small folia of which the more caudal ones have expanded laterally (3) The *culmen* is situated between sulcus postcentralis in front and fissura prima behind. Its median part shows five folia and its expansion on each side forming the anterior quadrangular lobule shows seven folia on the surface. The whole of the anterior lobe is to be considered as a median unpaired structure.

The middle lobe (Text-Figs 3, 4)—This is bounded in front by the fissura prima and caudally by the sulcus prepyramidalis. It consists of four subdivisions (1) The *simple lobule* is situated immediately behind the fissura prima. It is also a median unpaired structure though expanding laterally. It shows three folia on its exposed surface. It is limited caudally by the

postlunate fissure (post clival sulcus)   (2) The *median part* of the rest of the middle lobe includes the folium and tuber, each showing four folia on the exposed surface   (3) The *ansiform lobules* are paired portions of the
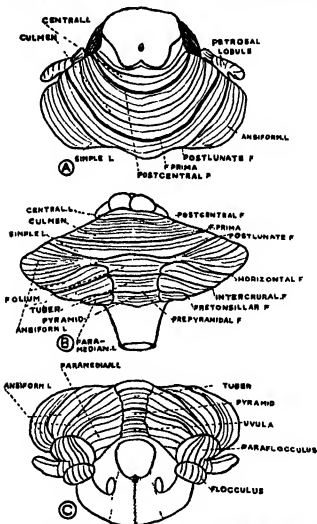
TEXT-FIG. 3.   Superior (A), Posterior (B) and Inferior (C) views of the cerebellum

hemispheres seen partly on superior view (Text-Fig 3 A) as the expansions external to the postlunate suclus and better seen on posterior and inferior views (Text-Figs 3 B, 3 C)   The ansiform lobules include the superior and inferior semilunar lobules and the bivental lobules   The superior and inferior semilunar lobules are separated from each other by the horizontal fissure   The superior semilunar lobule is larger and shows about nine folia exposed on the surface   The inferior semilunar lobule shows three folia exposed   Caudal to the semilunar lobule is the bivental lobule with the fissura intercruralis as the line of demarcation   The bivental lobules show three exposed folia   (4) The *paramedian lobules* are also paired parts of the middle lobe and are placed caudal to the ansiform lobules, the fissure pre-tonsillaris (retrotonsillaris of some authors) occurring between them   The paramedian lobules correspond to the tonsils
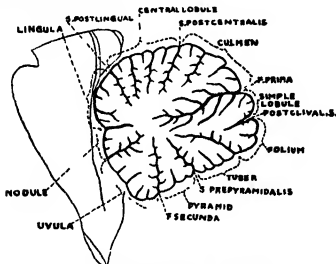
TEXT-FIG 4   Median sagittal section of the cerebellum
Drawing was made with camera lucida

The posterior lobe — This shows two subdivisions   (1) *The median part of the posterior lobe* includes the pyramid and uvula   The pyramid shows three folia and uvula, four surface folia   The pyramid is separated from the uvula by the fissura secunda and uvula from the nodule by the sulcus uvulo-nodularis   The uvula has no lateral extension or connection   But the sides of the cephalic folia of the pyramid apparently show a narrow connecting

band between them and the tonsils   (2) *The paraflocculus* forms on each
side the lateral parts of the posterior lobe   The paraflocculus is separated
from the ansiform and paramedian lobules by the sulcus parafloccularis and
from the flocculus by the sulcus flocculoparafloccularis   The paraflocculus
shows seven folia on its surface and has a small foliated petrosal lobule
prolonged from it

The flocculonodular lobe —This consists of the median part, nodule,
showing three surface folia and the paired flocculi showing seven folia on
the surface

Comment on the cerebellum —The cerebellum of entellus shows a better
development of the cerebellar hemispheres and a relative smallness of size
of the flocculus and paraflocculus compared with *Macacus silvicus* and
rhesus

Remarks on the Brain Stem

The anterior commissure of the entellus is relatively smaller than that
of *Macacus*   The optic chiasma, as in the case of other monkeys, lacks
the clear-cut appearance seen in man and appears relatively more bulky
The massa intermedia is a very broad union between the medial surfaces
of the two thalami   The pineal body is conical in shape   The corpora
mamillaria are distinctly seen as two rounded bodies, whereas in the brain
of *Macacus* this division into two is not seen   In the midbrain, the inferior
colliculi are smaller, more prominent and slightly more laterally placed
A section through the midbrain shows a large red nucleus   In the floor
of the fourth ventricle the stria acoustici are only faintly made out   The
colliculus facialis is well marked in the cephalic part of the paramedian
eminence   In *Macacus rhesus* the colliculus facialis is not prominent
(Hines, 1933)   In the lower part of the fourth ventricle, caudal to the trigo-
num hypoglossi and trigonum vagi, is a posterior funiculus

The pons is relatively very much larger than that of *Macacus*, and so
are the olives and pyramids   Thus there is a greater covering of the corpus
trapezoideum by the overlapping posterior border of the pons than occurs
in *Macacus* (Text-Fig 5)   The maximum width of the corpus trapezoideum
is only 2 mm in its lateral part   A greater development of the retro-
trigeminal part of the pons is indicated by the emergence of the fifth nerve
approximately midway between the two borders of the pons   This is a sign
of evolutionary advance (Abbie, 1934)   The facial and abducent nerves
emerge immediately below the pons   The acoustic nerve is an apparent
continuation of the corpus trapezoideum
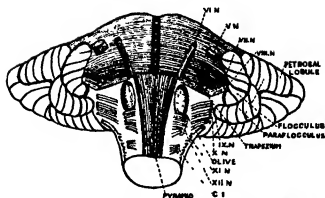
Text-Fig 5   Ventral view of pons, medulla and upper part of spinal cord

Summary and Conclusions

The brain of the Indian langur, *Semnopithecus entellus* is described The following are some of the significant observations resulting from a comparative study

(1) The cerebrum of *Semnopithecus entellus* compared with that of *Macacus* shows a less moulding of its inferior surface by the cerebellum

(2) The proportions of the weights of forebrain to mudbrain to hindbrain are 85 1 14 in *Semnopithecus entellus* and according to Tilney (1928), 84 2 14 in *Macacus rhesus*

(3) In its cerebral fissuration, especially in the regions of the inferior parietal lobule, occipital lobe, precuneus, parieto-occipital fossa, and collateral fissure, *Semnopithecus entellus* shows an intermediate stage between the other Cerecopithecidæ and *Hylobates*

(4) In the cerebellum of the langur there is a relatively greater development of the hemispheres and a lesser size of the flocculus and paraflocculus when compared with *Macacus*

(5) The entellus has larger olives, pyramids and pons and the corpus trapezoideum is covered over by the retro-trigeminal part of the pons to a greater extent than in *Macacus*

(6) This study of external morphology indicates that *Semnopithecus entellus* has probably the most advanced brain among the Cercopithecidæ A study of its internal structure is likely to be an extremely valuable addition to our knowledge of the Primate brain

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THE EGG-CAPSULE OF THE MILLIPEDE, THYROGLUTUS MALAYUS ATTEMS* (SYN THYROPYGUS MALAYUS CARL.)

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WHILE studying the ecology of the local millipedes, the author noticed that the eggs in case of *Thyroglutus malayus* are not laid in groups or masses but singly one in each small capsule As many as 31 egg-capsules have been obtained from a single female These capsules are made of earth and are so well designed and symmetrical in shape as to attract special attention from the surrounding soil

The general external outline of these capsules bears a superficial resemblance to a miniature fresh-water mussel, the two valves of which represent the two convex walls of the capsule These capsules have practically a uniform size, showing very slight variations in their dimensions The average measurements of the capsule are length 9 mm, breadth in the middle 6 mm, thickness, *i e*, distance between the two convex walls externally 5 mm, the curvature of the rim 10 mm

Fig 1

Photograph of five egg-capsules of *Thyroglutus malayus*
The cut capsules show the cavity and the egg *in situ*

* According to Attems the genus *Thyropygus* has been restricted and some of the species originally put under it have been transferred to his new genus, *Thyroglutus Thyropygus malayus* Carl is now synonymous with *Thyroglutus malayus* Attems

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The capsule has a very smooth surface on the inside (in contrast to the rough external surface), exhibiting the wonderful workmanship of the animal, that provides a velvety soft surface for the developing embryo If an egg-capsule is cut transversely in the middle, the egg is seen lying loosely in the cavity which is somewhat oval , the cut surface measures 5 mm × 6 mm The wall of the capsule in this region is 1 5 mm thick, so that the cavity is really 2 mm × 3 mm in cross axes This cavity does not extend right up to the two ends of the capsule but stops short by 2 mm at each tip These tips are merely solid portions of earth as can be clearly seen in a median longitudinal section of the capsule The entire space inside the capsule at the disposal of the developing embryo is more or less ellipsoidal with its axes measuring 5 mm , 3 mm and 2 mm

It has been observed in this case that during the process of egg-laying the tail and head ends of the animal are brought nearer to each other by the bending of the body As soon as the egg emerges out of the vaginal opening it is received by the open valves of the anal ring where part of the inner rectal membrane along with a drop of gelatinous fluid protrudes out through the anus. In some cases egg-capsule like pellet of earth is seen adhering to the gaping anal valves of the female millipede The egg capsule is shaped
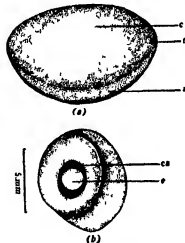
Fig 2

Diagrammatic sketch of the egg-capsule (a) entire capsule in latero-ventral view, (b) a capsule cut transversely in the middle to show the cavity and the egg c , convex outer surface, ce , cavity of the capsule, e , egg, r , rim of the capsule, t , tip of the capsule

according to the hollow of the anal valves where it is formed by the accumulation of more and more earth   The rim of the capsule is the portion which projects out when the anal valves containing the capsule are pressed together It has also been noticed that the female millipede spews a large quantity of mucus during the breeding period and this is spread over the capsule while it is being formed and is held in position between the anal valves   This mucus helps in sticking soil on to the external surface of the capsule

It is difficult to state the exact reasons for the formation of these egg capsules but it is definite that their formation is a physiological necessity as otherwise the eggs shrink and do not develop if even slightly exposed to the air   The capsule further protects the developing embryo from injury due to exposure or attacks of the enemies

The embryo with its developing appendages is set free by the breaking open of the capsule at one of its tips

It has also been possible to study the optimum egg-laying period in this millipede   The average temperature and humidity variations during this period are   maximum temperature 85°–95° F,   minimum temperature 62°–73° F,   humidity 65–95%   Observations extending over a period of three years show that the most suitable time of the year for egg-laying in this millipede is middle of September to end of October, but this period may also vary if the monsoon is late or feeble as in India the heat of the summer simply bakes the soil and makes it too dry for these delicate processes to take place

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CYTOGENETICAL STUDIES IN *DATURA*

I. Cytology of the Parents and of the F₁ Hybrid between *Datura fastuosa* and *Datura* sp.

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AND

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I Introduction

PLANTS belonging to the Solanaceæ have been, of all the families, the most widely investigated by the cytogeneticists Of the different genera, the genus *Datura* would appear to have attracted their attention only comparatively recently From the available literature, the earliest cytogenetical work on *Datura* seems to be Blakeslee's (1922), where he describes the variations in *Datura* as correlated to changes in chromosome numbers From that year onwards, a number of papers by Blakeslee and his co-workers on many species of *Datura* have been published The field that they have covered is wide, but mainly it can be said that they have studied mutants of *Datura*, either naturally occurring or artificially induced Haploids of *Datura*, chiefly of hybrid origin, have been studied by Satina *et al* (1937) Blakeslee and his co-workers (1936) published an account of investigation on *Datura*, where they have described the various methods by which the study of chromosomal differences between species of *Datura* can be made Stuart Gager

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and Blakeslee (1927) have described the nature of mutations in the chromo-
somes and among the genes, caused through exposure to Radium rays
Colchicine treatment and subsequent formation of chimeras with induced
polyploid numbers, have been studied in *Datura stramonium* by Satina *et al*
(1941) Chromosome deficiencies due to the above cause are dealt with in
a subsequent paper by Bergner *et al* (1941)

The materials for the present investigation are two of the locally avail-
able species of *Datura* The character contrasts in these two plants were so
marked, that these formed good materials for a study of the inheritance of
these characters A detailed description of the parental species and the
F_1 hybrid is given below The cytology of the parents and of the hybrid
is also described

II   Description of the parents and the hybrid

(a) Datura fastuosa (the black form), Pl II, A and B, 2

A large shrub with crookedly-branching very dark purple stems, growing
to 5–6 feet in height Leaves large, ovate, sinuate Flowers are solitary,
large, purple and nearly $8\frac{1}{2}$ inches long, on short pedicels Calyx, tubular
with five triangular lobes The base of the calyx is persistent Corolla
consists of five petals, gamopetalous, trumpet-shaped, purplish outside and
whitish inside, with plicate æstivation in the bud stage Stamens are also
five, filaments slightly shorter than the corolla, with long longitudinally
dehiscing anthers Ovary superior, bicarpellary, syncarpous, with fleshy
branched placenta and numerous ovules The wall of the ovary is thrown
into numerous closely-set papillæ, which become straight, sharp and stout
prickles, when the ovary ripens into a fruit The fruit is a loculicidally
septifragal capsule, big and ovally globose

(b) Datura sp (the white form), Pl II, A and B, 1

This parent is a short shrub growing to a height of 2–2½ feet It is
spreading in habit The stems are green in colour The flowers are smaller
than those of the other parent, of length varying from 6–6½ inches. The
colour of the corolla is yellowish-white In the fruits, the prickles are thinner
and shorter and less closely set than in the case of the other parents. The
fruits are globose

(c) The F₁ hybrid, Pl II, A and B, 3

This shows mostly characters intermediate between those of the parents
The plant grows erect and at the basal portion, the branches show a spread-
ing nature The stem is, however, dark-purple in colour and the average

height of the plant is about 4–4½ feet. The flowers are purple in colour and in length they are shorter than the black parent but longer than those of the white parent. The fruits show intermediate characters between those of the two parents.

There is nothing worth any special mention about the technique of crossing. The technique previously described for *Nicotiana* (Raghavan and Srinivasan, A. R., 1941 b) was followed.

III Cytological technique

Root-tips of the parents and the hybrid were obtained from plants grown in pots in the Botanical Gardens, Annamalainagar. These were fixed in Müntzing's modification of Navashin's fluid. Stages for meiotic studies were determined through acetocarmine examination and anther-smears were fixed in Belling's Navashin fluid. The fixed materials were embedded in paraffin following the usual schedule and sections were taken at thicknesses varying from 10–18 microns. Both the smears and the sections were stained in Haidenhein's iron-alum hæma-toxylin.

IV Genetical

The F_1 hybrid is intermediate between the two parents as regards most of the characters. However, a few features of the black parent are found to be dominant over those of the white in the F_1 generation. The following table shows the important features of contrasts between the parents and their expression in the F_1 generation.

TABLE I

Nature of the characters	Black parent	White parent	F_1 hybrid
Colour of the stem	Dark purple	Green	Dark purple
Habit	Tall, 7' and erect	Short, 3' in height and spreading	Tall and spreading, 4–5' in height
Length of the flowers	Long, 8½"	Short, 6"	8"
Colour of the corolla	Purplish outside and whitish inside	Yellowish-white	Purplish outside and whitish inside
Nature of fruits	Ovally globose, 2½" long	Round, 1½" long	2" long

Thus features of colour (of stem and of flowers) appear to be an expression of the dominance of the black parent over the white. Other features of the hybrid are intermediate.

V Cytology of the parents

(a) Cytology of Datura fastuosa (black)

Somatic chromosomes—Fig 1 represents the somatic metaphase plate of this parent The twenty-four chromosomes can be grouped under four types, which are as follows —

Type A—Longest chromosomes, 4 5 microns in length and with median constrictions

Type B—Slightly shorter chromosomes, 3 7 microns long and with sub-median constrictions

Type C—Still shorter ones, 3 microns long and possessing sub-terminal constrictions

Type D—Shortest chromosomes, with a length of 2 3 microns having sub-terminal constrictions

Out of the twenty-four chromosomes, two are of the A-type, six of the B-type, six of the C-type and the remaining ten of the D-type Other features such as secondary constrictions, satellites, etc, could not be seen Fig 2 shows the idiogram representation of the complement

Meiosis—Diakinesis (Fig 3)—During diakinesis, the twenty-four chromosomes form twelve bivalents A thorough examination of more than fifty diakinesis figures revealed that associations of more than two chromosomes were absent Hence multivalent formation can be said to be totally non-existent Of the twelve bivalents, one is large, possessing four chiasmata, two terminal and two interstitial This probably arises from the two longest A-chromosomes which have been found to have median constrictions. Two other bivalents have three chiasmata, one interstitial and two terminal Four ordinary ring-bivalents were met with, each having two terminal chiasmata The remaining five bivalents form a single terminal or subterminal chiasma each The total number of chiasmata in the black parent is twenty-three, of which four are interstitial Thus conjugation is complete and the formation of bivalents with more than two chiasmata presumably shows the close relationship of the parental chromosomes

First division—The nucleolus and the nuclear membrane disappear and the nucleus after a short prometaphase, enters upon the first metaphase stage (Fig 4) Twelve bivalents are arranged in the equatorial plate No associations were found between the bivalents Disjunction is normal

Second division—A short interphase intervenes between the two divisions, after which the chromosomes which disjoined in the first division

undergo homotypic division In the second metaphase stage (Fig 5) twelve chromosomes were seen at each of the two poles of the pollen mother cell The second division is normal and tetrads of both the iso-bilateral and the tetrahedral types are produced (Figs 6 and 7)
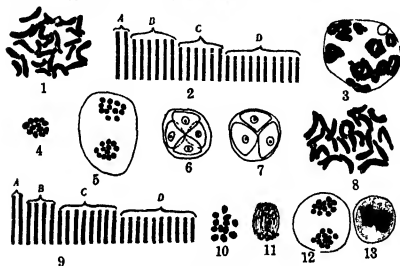
FIGS 1–13

FIGS 1–7 *Datura* (black)

Fig 1 Somatic plate. ×4400 Fig 2 Idiogram representation of the chromosome complement. Fig 3 Diakinesis ×4400 Fig 4 M I plate ×2200 Fig 5 Second metaphase ×2200 Figs 6 and 7 Tetrads ×1500

FIGS 8–13 *Datura* (white)

Fig 8 Diploid complement ×4400 Fig 9 Idiogram representation of the same Fig 10 First metaphase ×2200 Fig 11 Anaphase I ×1500 Fig 12 Second metaphase ×2200 Fig 13 Anaphase II ×1500

(b) *Cytology of Datura sp (white)*

Somatic chromosomes—The diploid complement of this parent is also made up of twenty-four chromosomes (Figs 8 and 9) There are two chromosomes of the A-type, four of the B-type, eight of the C-type and ten of the D-type There would appear to be difference only in the number of the two middle types of chromosomes (*i e*, B- and C-types of 3·7 and 3 microns length respectively) Whereas in the black parent there are six of each of these types of chromosomes, in the white parent, there are four of the B-type and eight of the C-type.

Meiosis—Twelve bivalents are formed by the twenty-four chromosomes as shown in Fig 10, which represents the polar view of the first metaphase plate So conjugation in this species also is complete Anaphasic separation (Fig 11) is regular in the first division and the disjoined chromosomes organize two nuclei during interphase During second metaphase (Fig 12) twelve chromosomes are seen at each of the two poles Second anaphase is also regular (Fig 13), and normal tetrads are formed

VI *Cytology of the hybrid*

Somatic chromosomes—The somatic chromosomes of the hybrid are represented in Figs 14 and 15 The four types into which the parental chromosomal complements were analysed, could be recognized here also In the somatic complement of the hybrid, there appear the two longest chromosomes, *i e*, of the A-type Similarly there were ten short chromosomes—*i e*, of the D-type—about 2 3 microns long, so that in these two types there is complete identity between the parents and the hybrid But in the nature of the two intermediate types there is some difference, for in the hybrid, we usually find only five chromosomes of the B-type (3 7 microns long) and seven chromosomes of the C-type (3 microns long) The different types of chromosomes and their number as occurring in the two parents and the hybrid, are shown in the following tabular statement

TABLE II

Chromosome length and nature of constriction	White parent $2n = 24$	Black parent $2n = 24$	F_1 hybrid $2n = 24$
A-type — 4 5 microns and median con strictions	2	2	2
B-type — 3 7 microns and sub-median constrictions	4	6	5
C-type —3 microns and sub-terminal constrictions	8	6	7
D-type — 2 3 microns and sub-terminal constrictions	10	10	10

The occurrence of five B-type and seven C-type chromosomes in the hybrid is as it should be, if we made a comparative study of the somatic complements of the parents that have entered into the formation of the hybrid complement The haploid complement of the white parent should consist of the following chromosomes one chromosome of the A-type, two of B-type, four of C-type and five of D-type Similarly the black

parent in its gametes should contain a genome composed of one chromosome of A-type, three of B-type, three of C-type and five of D-type As these two gametic chromosome sets together compose the diploid complement of the hybrid, the hybrid complement comes to contain two chromosomes of the A-type, five of B-, seven of C- and ten of D-types

Since the two gametic complements are identical to one another except in the number of the B- and C-types, we naturally find that in the hybrid these two types of chromosomes alone show a slight difference in their constitution Generally, the prevalence of these odd numbers of chromosomes amongst these types may be expected to cause some degree of asynapsis resulting in the formation of univalents But the difference between the B- and C-types is so slight that they may be expected to conjugate with one another without difficulty That is why, we find meiosis regular in the main The stray occurrence of trivalents which brings in its trail naturally the formation of univalents also, justifies this presumption

Meiosis—Stages earlier than diakinesis were not studied During diakinesis, the phenomenon of cytomixis was found to occur frequently It is interesting to note in this connection, that cytomixis was completely absent in the parents while it is so common in the hybrid This only goes to support the view expressed in a previous paper (Raghavan and Srinivasan, 1941 a), that this phenomenon is associated with hybridity Nevertheless apparently pure species have been found to exhibit this phenomenon as in the case of *Trida* (Raghavan and Venkatasubban, 1941) Less frequent occurrence of cytomixis has been recently reported in the species, *Guettarda speciosa* (Raghavan and Srinivasan, 1941 b) and *Portulaca tuberosa* (Raghavan and Srinivasan, 1941 c) Nandi (1937) has recorded cytomixis during diakinesis stages in *Oryza* and is of opinion that it leads to polyploid gamete production through the formation of bi-nucleate pollen mother cells X-rayed materials of pure species show the phenomenon of cytomixis to an extreme degree, e g , *Capsicum* (Raghavan and Venkatasubban, 1940)

Cytomixis in the present case is very interesting In Fig 16, nuclear extrusion takes place into a single cell from two adjacent cells In Fig 17, nuclear matter from a single cell is extruded at the same time into two cells on either side of it This recipient cell is found to transfer its chromatic material to the next cell and so on Normal cytomixis between two adjacent cells has also been met with (Fig 18) In the prometaphase stages also this phenomenon was found to occur (Fig 19) Fig 20 shows pollen mother cells at first metaphase stage, one of the metaphase plates showing a tendency to migrate The relegation of two of the bivalents to a peripheral position

while the rest occupy the equatorial plate (Fig 25), may also be regarded as showing an abortive attempt at cytomixis In the present case the extent to which cytomixis occurs decreases as the pollen mother cells advance into the later stages.
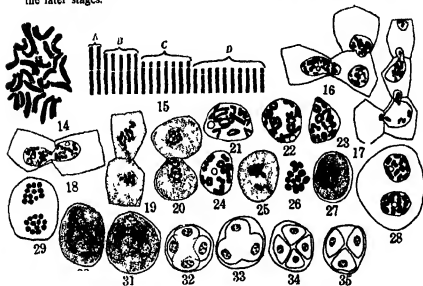
FIGS 14–35   Cytological stages of the F₁ hybrid

Fig 14   Somatic plate ×4400 Fig 15   Idiogram representation of the same Figs 16–20 Stages of cytomixis ×1500 Figs 21–24   Diakinesis stages In Fig. 23 a trivalent and a univalent are shown All the figures to the magnification ×2200 Figs 25 and 26   Metaphase I ×2200   Fig 27 Anaphase I ×1500 Fig 28 Interphase ×2200 Fig 29 Second metaphase ×2200 Figs 30 and 31 Second telophase ×1500 Figs 32–35 Furrowing and tetrad formation ×1500

Normal diakinesis was also quite common   Fig 21 shows a P M C at early diakinesis in which the synapsing chromosomes are long and slender   Of the twelve bivalents, three are of the ring-type, each with two terminal chiasmata   All the other bivalents have one terminal chiasma each   At a later stage, the chromosomes shorten and thicken (Fig 22)   In this case one of the bivalents was found to have an interstitial chiasma   Three ring bivalents were met with here also   In one case, a trivalent and a univalent were found along with ten bivalents, but such cases were very rare (Fig 23)   At later diakinesis, the configuration of the bivalents becomes clear (Fig 24), when three ring bivalents and nine rod bivalents

are observed Thus during diakinesis stages the meiosis shows normal conjugation between the chromosome sets of the parents The total number of chiasmata is fifteen, interstitial chiasmata being almost absent

At first metaphase all the twelve bivalents are arranged in the equatorial plate (Fig 26) Very occasionally however, one or two of the bivalents are found to be located away from the equatorial plate (Fig 25) First anaphase is regular but for the somewhat late disjunction of one of the bivalents (Fig 27)

Interphase follows the first division and the nucleus assumes a globular appearance (Fig 28) The constricted appearance of the chromosomes is characteristic of the interphase nuclei The chromosomes are peripherally disposed at equal intervals from each other The second prometaphase follows, but is of very short duration Soon the nuclear membrane disappears and these constricted bodies contract and enter upon the second metaphase Twelve chromosomes at each end could be recognized as seen from the pole (Fig 29) During second telophase, the chromosome groups are arranged either in the iso-bilateral or in the tetrahedral pattern (Figs 30 and 31 respectively)

Quadripartition of the tetrads is through furrowing (Figs 32 and 33) The method of formation of iso-bilateral tetrads conforms to that described for *Nicotiana glutinosa* (Raghavan and Srinivasan, 1941 *a*) Normal tetrads of both iso-bilateral and tetrahedral types are formed

VII Discussion

Interspecific hybrids between parents with the same number of chromosomes are not rare These are interesting in the variety of genetical and cytological features that they present to us, such as, fertility or otherwise of the F_1 hybrids, chromosome conjugation, abnormalities of meiosis, etc There is a gradation from fully fertile hybrids to completely sterile ones This only shows that the mere identity in the number of chromosomes is not enough by itself for the complete pairing of chromosomes The degree of fertility is in almost all cases, determined by the degree of conjugation between the parental chromosomes In completely sterile hybrids, either conjugation is absolutely lacking, or weak conjugation takes place to such a low extent, as can be considered to be no better than asynapsis Hybrids of this latter-mentioned type are many Ramanujam (1937) obtained a hybrid between *Oryza sativa* ($n = 12$) × *O officinalis* ($n = 12$), in which during diakinesis twenty-four unpaired chromosomes were met with In some cases loosely formed bivalents were found and that too only to a low degree This hybrid was found to be sterile Crosses in *Nicotiana*, such

as those between N sylvestris $(n = 12) \times N$ glutinosa $(n = 12)$, N rustica $(n = 24) \times N$ tabacum $(n = 24)$ and N Raimondii $(n = 12) \times N$ glauca $(n = 12)$ have led to similar results (Goodspeed, 1934)

Partial pairing between chromosomal sets of the parent species has been observed in other cases Hybrids between species of Lactuca (Whitaker and Thompson, 1941), L. tatarica $(n = 9) \times L$ indica $(n = 9)$ showed $7_{11} + 4_1$, while F_1 of L sativa $\times$ L vivosa (both $n = 9$) showed $8_{11} + 2_1$ In spite of such partial pairing, the hybrids were found to be sterile In hybrids of Nicotiana (Goodspeed, 1934), Crepis (Babcock and Emsweller, 1936), Aegilops (Perceval, 1930) and Gossypium (Webber, 1935), such partial pairing between parental chromosomes resulting in varying numbers of bivalents and univalents were met with

In the present case however, the parental chromosome sets seem to be completely homologous to each other This is proved by the fact that twelve bivalents are regularly formed But the number of chiasmata in the diakinesis stages of the hybrid is far less than in the parents This probably signifies that, after all, two different parental sets of chromosomes—however homologous they may be—cannot pair as fully as those of the same parental species In the present case, the average total number of chiasmata (during the mid-diakinesis stages) in the parental species is about twenty-three, of which four are of the interstitial type, the rest being terminal In the hybrid, on the other hand, the number of chiasmata at about the same stage of meiosis, is about fifteen, all of which are terminal Only very rarely do we see an interstitial chiasma There is thus a marked decrease in the number of chiasmata in the hybrid, as compared to that of the parents, Also, interstitial chiasmata are conspicuous by their absence in the hybrid Similar observations would appear to have been made by Goodspeed (1934) in interspecific hybrids of Nicotiana and his interpretation on these phenomena seem to indicate a correlation between these and the degree of chromosomal identity in the parental species He says that " with increase in the number of bivalents (i e , in the homology of the parental chromosome sets), there is a disproportionately greater increase in the total number of chiasmata, because in certain of the bivalents two chiasmata are formed, and there is also an increase in the proportion of interstitial chiasmata With almost complete pairing, the total number of chiasmata approaches that in the parent species " Thus according to Goodspeed, the greater the number of chiasmata formed, the more homologous are the parental chromosomes Viewed in this light, the chromosome sets of the two parents in the present case cannot be regarded as being so completely homologous.

For a casual observer the two parents used in the present cross, may appear to be only varieties of a distinct fundamental species On closer scrutiny however, we find that the external features, such as the size and nature of the vegetative and the reproductive parts do not go to support this assumption The large differences that exist between the two parents may, by themselves, be sufficient to classify them as distinct species In addition to this morphological evidence, we have got cytological data also to prove the relative distance between the two parents For example, a study of the somatic complements shows some differences in the chromosome morphology of the two parents These differences have been pointed out already Another, fact to reckon with, is that the number of chiasmata in the hybrid is much less than in the parents themselves, also interstitial chiasmata are characteristically absent in the hybrid This, as has been pointed out already, suggests the somewhat distant relationship between the two parents It will not therefore be wrong to regard them as two distinct species

In spite of the above-mentioned differences, there is no deficiency in the homology between the parental chromosomes Most probably the genes of the chromosomes are related to each other Consequently, the meiosis is almost as normal as in the parent species and the F_1 hybrid is fertile Such fertile interspecific hybrids were met with in the case of *Nicotiana*, between *N solanifolia* ($n = 12$) × *N paniculata* ($n = 12$) (Goodspeed, 1934) Hybrids of *Lactuca*, other than those mentioned above, were found to be fertile, though to a lesser extent than the parents (Whitaker and Thompson, 1941)

Taxonomical relationships have recently been determined through studies in the chromosome behaviour of interspecific hybrids Species whose chromosome sets pair freely with each other, are believed to be nearly related, the only difference between their chromosomes being of the nature of certain genetic factors Partial or no pairing has been taken to represent distant relationship of the parents In *Nicotiana* this principle has been found to be true Thus, Goodspeed (1934) remarks " when two chromosome complements are capable of co-operating so that a normal soma is built, these chromosome sets have at least residual homology and presumably contain genes in common " He also says that " there is no justification for a disbelief in the significance of pairing as indicative of relationships in *Nicotiana*, because in other materials the evidence at present may not warrant such a conclusion " We find that this principle is applicable to the present hybrid in *Datura* No abnormality of any serious nature having been observed in the hybrid, the two species may be looked upon as being very

nearly related to each other and can be traced back to identical origin So, the above said principle that a close correlation exists between chromosome pairing and taxonomic relationship, is found to be true in the case of *Datura* also

However cases have been found where this principle presents obvious difficulties Crosses by Clausen (1931) in *Viola* are examples of this *Viola arvensis* ($n = 17$) was crossed with *V tricolor* ($n = 13$) and the F_1 hybrid showed $13_{11} + 4_1$ according to the Drosera type of chromosome conjugation Thus the thirteen chromosomes of *V tricolor* are homologous to thirteen chromosomes in the haploid set of *V arvensis* Another cross between *V tricolor* ($n = 13$) and *V nana* ($n = 24$) also showed perfect pairing between the *tricolor* and the *nana* chromosomes If the hypothesis that chromosome pairing is indicative of the relationship between species be true, thirteen chromosomes of *V arvensis* which paired with the chromosomes of *tricolor*, should conjugate with the thirteen chromosomes of *V nana*, with which also the *tricolor* chromosomes exhibited pairing This however was not the case and two to six bivalents were only formed in the hybrid between *V nana* and *V arvensis* Even this bivalent formation might have been due to autosyndesis In *Nicotiana degluta* where the chromosomes are obviously homologous, non-pairing has been reported (Müntzing, 1935) Ramanujan (1937) is of opinion that chromosome conjugation may be influenced by genetic and environmental factors According to him "while conjugation of chromosomes indicates a kind of homology between them, non-conjugation does not necessarily always mean non-homology"

The above instances show that much caution should be exercised while interpreting partial or no pairing with reference to relationship between parents of interspecific hybrids But there seems to be no difficulty in interpreting complete pairing (as in the present case) as indicative of taxonomic nearness of the parental species

VIII Summary

Two species of a local *Datura* were crossed and the hybrid compared with the parents

The somatic chromosome complements of the parents were analysed and compared with that of the hybrid

Meiosis in the parents and the hybrid is also described Chromosomal pairing in the hybrid is discussed as throwing light upon the relationship between the parents

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A   Photographs of whole plants of 1 The white parent   2 The black parent
and 3 The F₁ hybrid

B   Photographs of flowers and fruits of 1 The white parent
2 The black parent ; and 3 The F₁ hybrid

NEW CONCEPTS OF THE SOLID STATE*

By Sir C V Raman

My first duty is to thank the authorities of the Nagpur University on behalf of the Indian Academy of Sciences for the invitation which has enabled the Annual Meeting of the Academy to be held this year under the auspices of the University The Fellows of the Academy deeply appreciate the labours of the Chairman and members of the local Committee and of Prof Moghe in making the arrangements for the meeting and are grateful for the hospitality which has been generously provided for the occasion

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For good or for evil, we live in an age of science No one who is familiar with the history of science would fail to recognize the great influence which has been exercised on its progress by the work of the various national academies of science, as for instance the Royal Society of London and the Academy of Sciences at Paris The publications of these academies are the primary records of scientific discovery and invention in their respective countries To no small extent, also, the Academies have been responsible for the promotion and encouragement of research work and for the co-ordination of the research activities of the Universities During the seven years the Indian Academy of Sciences has been in existence, it has striven to fulfil these functions in our country The *Proceedings* of the Academy which have appeared punctually, month after month, embody the best part of the research work done in most of the Indian Universities. It is greatly to be desired that these Universities appreciate what the Academy is doing for them and help the Academy to carry on under the present difficult conditions

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I propose to devote my address this year to an exposition of the new ideas concerning the solid state of matter which have emerged from recent investigations made at Bangalore The vast majority of actual solids are crystalline in structure and are either single crystals or else consist of polycrystalline aggregates The gateway to an understanding of the solid

state is therefore to be found in the study of crystals   The most effective starting point for such a study is, again, the ultimate structure or atomic architecture of the solid   The physics of the solid state of matter indeed concerns itself largely with the relationship between the atomic grouping in space which characterizes a crystal and the physical behaviour of the solid in various circumstances

As is well known, crystals often possess beautiful external forms with specific geometric features   The symmetry characters of these geometric forms stand in the closest relation to the physical properties of the solid, such relationship being most evident when we consider those properties which vary with direction   The study of the geometric forms and of the physical properties of crystals resulted in the classification of crystal forms into six or seven systems and their further sub-division into thirty-two classes of crystal symmetry   It is natural that crystallographers were led by such studies also to speculate on the features in the internal architecture of crystals to which could be ascribed the external symmetry properties manifested by them   The theoretical investigations which dealt with this problem resulted in the recognition that a crystal is essentially a repetitive pattern in space and that the material particles of the solid are arranged in regular geometric order in a three-dimensional space-lattice   The discovery of the 14 possible kinds of space-lattice and of the 230 possible ways of grouping the atoms, each in its own appropriate type of space-lattice and coming under one or another of the 32 possible symmetry classes, gave the necessary precision and completeness to such general notions of crystal architecture

The ideas of the mathematical crystallographers of the nineteenth century found a spectacular confirmation in Laue's great discovery made in 1912 of the diffraction of X-rays by the space-lattice of crystals   During the thirty years which have elapsed since that discovery, a vast amount of detailed knowledge regarding the structure of individual crystals has been built up by the labours of the X-ray crystallographers   Around such knowledge, again, there has been a great deal of discussion regarding the nature of the forces which held together the atoms, ions or molecules in a crystal in the form of a coherent solid

It must be recognized that the concept of a regularly ordered assemblage of atoms, ions or molecules in a space-lattice is only a static description of crystal structure and does not suffice to give a complete view of the solid state   That the density and many other physical properties of

a solid vary with temperature is clear indication that the atomic positions in a crystal are subject to disturbance by thermal agitation A description of the possible atomic movements in a crystal is thus as important for crystal physics as a knowledge of the static structure In other words, a dynamic picture of the crystalline state is required as a complement to the static picture furnished by the space-group theory The possible modes of atomic vibration would evidently be determined by the atomic groupings in the crystal lattice and the forces that come into play when such grouping is disturbed It follows that the static and dynamic aspects of crystal architecture should stand in the closest relationship to each other

A dynamic concept of the solid state is necessarily the starting point in any consideration of the thermal properties of a crystal, e g , its specific heat, thermal expansion or thermal conductivity It is equally fundamental in any attempt to elucidate such physical properties of solids as are notably influenced by temperature, e g , the electrical resistivity of metals The subject of crystal dynamics assumes a special importance in considering the effects arising from the propagation of electromagnetic waves through crystals, e g , the scattering of light or the diffraction of X-rays Spectroscopic and X-ray studies on crystals indeed afford us a penetrating insight into the problems of the solid state

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The theorists who have handled such problems in the past have proceeded by carrying over notions derived from the classical theory of vibrating elastic solids into the domain of atomic dynamics The history of physics during the present century suggests that all such extrapolations from macroscopic to atomic concepts must be regarded with caution The extrapolations made in the Debye and Born theories of crystal dynamics do not, however, appear to be justified even from a purely classical point of view It is not surprising, therefore, that the conclusions derived from these theories fail to survive the test of comparison with the experimental facts in several different branches of research Before we proceed to consider evidence of this kind, it appears desirable to examine the foundations on which these theories rest

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We may, in the first instance, comment on the well-known specific heat theory of Debye which has had the run of the text-books of physics for many years and even yet seems to be in favour The theory assumes that the thermal energy of a solid may be identified with the energy of elastic waves travelling within it, and gives an expression for the energy in terms

of the velocities of these waves That these assumptions are unjustifiable
is evident from Debye's own formulæ For, the calculation shows that
a very large proportion of the elastic vibrations must be assumed to possess
wave-lengths comparable with the lattice spacings of the crystal Their
frequencies also become comparable with those of the vibrations of the
individual atoms Even according to the classical principles, vibrations of
such short wave-lengths and high frequencies could scarcely be expected
to travel through the crystal with the assumed acoustic velocities Indeed,
the familiar fact that thermal energy does not travel at all but only diffuses
with extreme slowness in solids is a clear disproof of the basic assumptions
of the Debye theory Far from supporting the postulates of the theory,
the facts point to exactly the opposite conclusion, namely that no sensible
part of the thermal energy of solids consists of the elastic vibrations of
macroscopic physics

The so-called postulate of the " cyclic lattice " on which the crystal
dynamics of Born is based was introduced by him as a mathematical device
to escape the difficulties which he believed to arise from the unspecified
conditions at the external boundary of the crystal The postulate in effect
prescribes " wave-lengths " for the atomic vibrations in the crystal which
bear no relation to its internal architecture but are related to its external
dimensions in exactly the same way as the elastic vibrations of macro-
scopic physics The postulate of the cyclic lattice has no theoretical justi-
fication and its introduction makes Born's approach to the problem of
crystal dynamics wholly unreal and no less open to criticism than the
theory of Debye

The fallacy of the basic ideas underlying the Debye and Born theories
becomes evident when we consider the nature of the vibrations within
a solid indicated by the classical theory of elasticity The form and size of
the external boundary of the solid determines the possible modes of elastic
vibration In each individual vibration, the motion at all points within the
solid has a specifiable frequency and a coherent phase-relationship But
there would be an immense number of such modes with varying frequencies
The superposition of all such modes, assumed to be co-existent, would
therefore result in the agitation within the solid being of a completely chaotic
character, varying from point to point and from instant to instant without
any recognizable periodicity in space or recurrence in time Thus, in effect,
the assumptions made in the Debye and Born theories are equivalent to the
assertion that while the static arrangement of the atoms in a crystal is
one of perfect order and regularity, the dynamic character of their move-
ments is one of perfect chaos and disorder, indeed exactly of the same

kind as the movements or vibrations of the molecules of a gas This conclusion is obviously so improbable that we may well feel justified in rejecting without hesitation the premises on which it is based

A crystal, as we have seen, is a periodic array of similar particles, similarly situated and capable of influencing each other's movements It follows that the vibrations of such an assemblage should exhibit a high degree of orderliness, approaching the ideal of a perfectly co-ordinated vibration in which the frequency, amplitude and phase are identically the same throughout the crystal To picture such a vibration, we may first consider the group of the atoms present in an individual cell of the space-lattice The internal vibrations of such a group would comprise several distinct modes determined by the number of atoms present Each such vibration may then be pictured as occurring in identically the same way in every cell of the crystal lattice Geometrically, such an oscillation could be represented as a periodic movement, relative to each other, of the inter-penetrating simple lattices of similarly placed atoms of which any crystal may be regarded as built up Such a vibration would have a uniquely definable frequency, and the vibration spectrum of the crystal would therefore consist of a finite number of discrete monochromatic frequencies

Thus, instead of an infinite array of chaotic movements varying arbitrarily in phase from cell to cell of the crystal, and having a continuous spectrum of frequencies, we obtain a finite group of vibration modes with space-patterns coinciding with the lattice structure of the crystal and having a set of discrete monochromatic frequencies These vibrations are essentially periodic changes in the fine structure of the crystal and do not involve mass movements of the substance of the solid. Hence, neither the existence of an external boundary nor the conditions restraining its movements can have any influence on such vibrations

The most appropriate choice for the space unit of the three-dimensional repetition-pattern of the atomic vibrations is evidently that which enables all the modes possible to be included without redundancy Hence the appropriate choice is not the cell having the smallest dimensions or including the least number of atoms, but one which is fully representative of the crystal structure and symmetry In the majority of crystals, the number of atoms included in such a space-unit would be fairly large Hence, the internal vibrations of the group of atoms contained in it would comprise the largest proportion of the available degrees of freedom of movement, indeed all except a small residue representing the translatory movements

of the chosen cell   To enable these latter to be included in the scheme, we may consider the internal vibrations of a group of atoms contained in the cells of a super-lattice having cells of twice the linear dimensions and therefore of eight-fold volume   Proceeding in this way by successive steps, the vibration spectrum of the crystal could be developed with all desirable completeness as a set of monochromatic frequencies

*   *   *

It will be realised that the geometric characters as well as the frequency distribution of the atomic movements in crystals obtained in this way would be radically different from those indicated by the Debye and Born theories   It is evident also that the new concepts involve striking differences in the spectroscopic, X-ray and thermal behaviour of crystals as compared with those derived from the older ideas   The issues arising between the new and the older concepts are thus capable of being brought to an exact experimental test

*   *   *

The atomic vibrations in crystal lattices are accessible to optical and spectroscopic investigation in several different ways   A method which makes the entire frequency range conveniently accessible to observation is the spectroscopic study of the scattered radiations emerging from a crystal traversed by monochromatic light   The most striking feature revealed by such studies with crystals is the extreme sharpness of the displaced lines appearing in their spectra   Even in those cases where the lines are somewhat diffuse, they sharpen into the finest lines when the crystal is cooled down to low temperatures   The monochromatism of the lattice frequencies thus indicated is especially significant when the vibrations are observable only in the crystalline state, in other words when the lines disappear in the molten or dissolved material   These facts are wholly inconsistent with the Debye and Born theories   Indeed, it may be said that the character of the spectra observed even with the simplest of crystals bears no resemblance to the diffuse continua suggested by these theories   Evidence confirmatory of the new concepts of crystal dynamics is also furnished by the absorption and luminescence spectra of crystals observed at low temperatures, e g, diamond   Here again, the lattice spectrum is revealed as a set of discrete monochromatic frequencies stretching down to low values, in startling contrast with the conclusions of the Debye and the Born theories

*   *   *

As already explained, the new concepts indicate a close correspondence between the static structure and the dynamic behaviour of a crystal,

in other words that the atomic vibration patterns are either identical with or closely related to the lattice structure of the crystal As an immediate consequence of this relationship, it follows that the lattice planes of a crystal should give two distinct types of X-ray reflection—a dynamic reflection with altered frequency in addition to the static reflection of unmodified frequency discovered by Laue The more perfectly co-ordinated is the oscillation of the lattice structure, the more perfect would be the geometric character of the dynamic X-ray reflections Hence, these reflections should be shown in the most striking way by diamond-like structures in which the entire crystal is practically a single molecule and less perfectly by other crystals in which the lattice structure is of a more open kind

That the lattice planes in crystals do give the new type of dynamic X-ray reflection here indicated and that such reflections are incapable of being explained on the older theories was discovered and announced by myself and Dr Nilakantan in March 1940 In a symposium of fifteen papers published in the *Proceedings* of the Academy for October 1941, the theory of these new X-ray reflections, their relation to quantum mechanics and the experimental facts as observed with diamond and numerous other crystals has been thoroughly explored It has been proved that the experimental facts are, on one hand fatal to the Debye and Born theories and that on the other hand, they give the strongest support to the new concepts of the solid state

To the pioneer investigations of Einstein, we owe the basic principles of the quantum theory of the specific heat of solids He showed clearly that the thermal energy of a crystal stands in the closest relation to its optical properties and could, in fact, be expressed in terms of the characteristic frequencies of atomic vibration appearing in the infra-red region of frequency In his earliest paper, Einstein suggested that the atomic frequencies could be assumed to be monochromatic Considering one such characteristic frequency in the case of diamond, he evaluated the same from the specific heat data It will be seen from our present discussion that the basic assumption of monochromatism was justified, and that the only amendment needed in Einstein's theory was the inclusion of the full number of discrete monochromatic frequencies demanded by the lattice structure of the crystal with the appropriate statistical weights It is also seen that the application of the macroscopic theory of elastic vibrations due to Debye, successful though it seemed at the time, was, in reality, a false step

In a symposium of seven papers published in the *Proceedings* of the Academy for November 1941, the problem of the thermal energy of crystalline solids has been discussed fully from the new point of view and compared with the experimental data for a variety of substances. In several cases where the necessary spectroscopic data were available, these have been effectively made use of. In other cases, *e g*, metals, the specific data themselves have been utilised to evaluate the atomic frequencies. The most significant fact which emerges from the symposium is that the experimental facts in several cases which refused obstinately to fit into the Debye and Born theories find a natural explanation in the new concepts without the aid of any special hypothesis

Summary

The postulates on which the Debye theory of the specific heat of solids and the Born crystal dynamics are respectively based have been critically examined and shown to be theoretically untenable. Since a crystal is a three-dimensionally periodic grouping of similar oscillators coupled together, it follows that the modes of vibrations possible would be also space-periodic, the geometric modes being determined by the characters of the atomic space-grouping in the crystal. They would further form a finite and enumerable set of monochromatic frequencies. The spectroscopic, X-ray and thermal behaviours of a crystal would on these views be radically different from those consequent on the Debye and Born theories. The experimental facts are found to contradict the conclusions of these theories and on the other hand, to be in full accord with the new concepts

A CONTRIBUTION TO THE LIFE-HISTORY OF *VAHLIA VISCOSA*, ROXB, AND *VAHLIA OLDENLANDIOIDES*, ROXB

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CONTENTS

I Introduction

THE family Saxifrageæ is a fairly well worked family It must be described as a heterogeneous family for the various genera of this natural order exhibit a variety of characters, and the systematic position of some of the genera has been questioned , for example, Pace (1912) having studied the life-history of a few species of *Saxifraga*, *Parnassia palustris* and *Drosera rotundifolia*, is of the opinion that *Parnassia* has greater affinities to *Drosera* than to *Saxifraga* and concludes that it should be included in the Droseraceæ Dahlgren (1930) gives an account of the development of the endosperm in a number of

genera of the Saxifragaceæ and draws a scheme of endosperm development in five genera, where he distinguishes three important types of endosperm development, namely, nuclear endosperm, cellular endosperm and an intermediate type in which both kinds of endosperm development are combined Chapman (1933) gives a summary of the more important of the work already done in the family In 1933, Mauritzon made one of the best contributions to our knowledge of the development of the embryo-sac endosperm and embryo in a number of genera of the Saxifragaceæ In a critical study of the closely allied families of Crassulaceæ and Saxifragaceæ, he classifies the various genera of the Saxifragaceæ into two broad groups, the ' Krassinucellate " and the " tenuinucellate ", on the basis of the nature of the nucellus Among the tenuinucellate, there are two groups, those in which there is a single layer of nucellar cells and those in which the nucellus is two cells thick just above the megaspore mother cell He found that some genera were characterised by the possession of a single integument, while the others had two integuments He also describes the nature of the ovary and ovule, and gives the developmental stages of the embryo-sac, the endosperm as also the embryo in a a number of genera Like Dahlgren (1930), Mauritzon (1933) also groups the various genera investigated till then under three heads, mentioned by Dahlgren (1930), the basis of grouping being the nature of endosperm development

So far as we are aware the work on the genus *Vahlia* is very meagre Skovsted (1934) gives the haploid chromosome number of *Vahlia oldenlandioides* as six which number is confirmed in the present investigation Mauritzon (1933) has given a few stages in the development of the embryo-sac, the endosperm and the embryo in *Vahlia oldenlandioides* No reference could be found in the available literature to any study of the species *Vahlia viscosa,* Roxb The present paper describes the entire life-history of *Vahlia viscosa,* and also that of *V oldenlandioides,* for purposes of comparison

II Material and Method

Plants of *Vahlia viscosa* and *V oldenlandioides* were grown in the University Botanical Gardens, Annamalainagar Different stages of ovary were fixed in corrosive sublimate formalin-Acetic-Alcohol fixative For determining the chromosome number, flower buds after acetocarmine examination (to see whether they showed pollen mother cell division stages) were fixed in hot corrosive sublimate-formalin-Acetic-Alcohol fixative Sections were cut at thicknesses varying between 6 and 14 microns All the materials were stained in Haidenhain's Iron-alum Hæmatoxylin

III *Vahlia viscosa* Roxb

(a) Floral Organogeny —Flowers occur usually in pairs (Fig 4, A and B) in the axils of leaves Each flower is initiated as a small conical protuberance (Fig 4, A) The sepals are the first to be initiated Each sepal arises as a fold from the sides of the conical body (Fig 1, *se*) The flower primordium, which to begin with, has a convex free end at the time of the differentiation of the sepals becomes flattened and in later stages becomes progressively depressed, in the centre The next floral organ to be initiated is the stamen and not the petal The stamens arise as protuberances from near the rim of the depression or cavity which is being formed in the centre of the free end of the thalamus (Fig 2, *st*) The primordia of the petals appear between the stamens and the sepals (Fig 3, *pe*) By this time, the depression in the free end of the thalamus is very deep This is the ovarian cavity and from its sides near the top, twoprot uberances arise (Fig 3, *ca*) These are the placenta They grow downwards into the ovarian cavity into which they hang When they are fully formed, the ovules appear as papillate protuberances from all round the two placentas There can be no doubt that the gynæcium is made up of the fusion of two adjacent carpellary margins This is quite clear when transverse sections are taken of very young gynæcia (Fig 7) where the bi-carpellary nature of the ovary is quite evident especially at the top The two carpel ends have not fused completely so that their individuality is unmistakably clear At a later stage, they fuse together to form a single-celled ovary with two parietal placentæ (Fig 6) On account of the peculiar disposition of the placentas the latter appear as two oval bodies unconnected with either one another or with the wall of the ovary, if a transverse section of the ovary is taken in the middle region That the gynæcium is bi-carpellary and the placentation parietal, there can be no doubt about, as sections near the top of the ovary reveal the clear fusion of the adjacent carpellary margins It is likely that the two carpellary margins by whose fusion the parietal placentas are primarily formed, have failed to form any such normal placentas except at the top of the ovary where on account of the massiveness of the placentas so formed and the concentration in one region, they hang as it were into the ovary cavity In Fig 5, a transverse section taken at level A, may reveal a position as depicted in Fig 7, but a section taken lower down will present Fig 6

(b) Microsporogenesis —Transverse sections of very young anthers reveal the primary archesporium The primary archesporium is hypodermal and usually consists of a plate of two cells (Fig 8) The primary wall cell cuts off towards the periphery a layer of primary parietal cells and a layer of primary sporogenous cells towards the interior The primary parietal

cell again divides twice periclinally As a result, the anther wall including the epidermis becomes four cells thick (Fig 9) The innermost layer of the parietal cells functions as the tapetum The tapetal cells are of differing
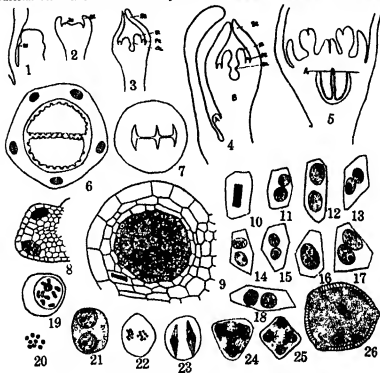
FIG 1 26 *Vahlia viscosa* Roxb

Fig 1 Origin of sepal at *s* ×150 Fig 2 Origin of stamens at *st* ×150 Fig 3 Origin of petal at *pe* ×150 Fig 4 Shows the origin of two flowers A and B in the axil of a leaf, and also that of the carpels at *ca* ×150 Fig. 5 L S of ovary showing the pendulous parietal placentas ×150 Fig 6 T S of ovary showing uni-locular and bi-carpellary ovary Five vascular strands are seen along the fruit wall ×150 Fig 7 T S of very young gynecium showing the bi-carpellary nature of the ovary ×150 Fig. 8 Hypodermal archesporium of two cells in the anther ×800 Fig 9 Shows the anther-wall is four cells thick the innermost of which is the tapetum ×1500 Fig 10 Mitotic division of the tapetal nucleus ×2200 Fig 11 Bi-nucleate tapetal cell ×2200 Figs 12-18 Various stages in the formation of the plur-nucleate tapetal cells ×2200 Fig 19 Pollen mother-cell nucleus in diakinesis, nine rod bivalents are present. ×2200 Fig 20 Metaphase I *n* = 9 ×2200 Figs 22 and 23 Polar and side view respectively of Metaphase II ×2200 Figs 24 and 25 Tetrahedral and isobilateral arrangement of the pollen tetrads ×2200 Fig 26 Two-celled mature pollen grain Note the three germ pores ×2200

sizes and stain brightly, and to begin with are uni-nucleate (Fig. 9) Often small vacuoles appear in the tapetal cells The pollen mother cells which are closely packed together are surrounded by the tapetal layer When the pollen mother cells are in the early prophase, the nuclei of the tapetal cells divide and become bi-nucleate (Fig 11) This division is distinctly mitotic (Fig 10), the mitotic nature of the division of the tapetal nucleus has been observed in a number of families like Capparidaceæ (Raghavan, 1938), Scrophulariaceæ (Srinivasan, 1940), Acanthaceæ (Rangaswamy, 1941), etc This is followed by a number of nuclear divisions and their immediate fusion, as a result of which the tapetal cells become pluri-nucleate (Fig 15) Various stages in the formation of the pluri-nucleate condition have been noticed (Figs 12–18) The possible significance of this has been discussed recently by Raghavan and Srinivasan, A R (1941) Fig 19 represents the pollen mother cell nucleus in the diakinesis stage Nine bivalents, all of which are of the rod kind can be clearly seen two of them are attached to the nucleolus (Fig 19) In Fig 20 is represented the polar view of metaphase I, and nine bivalents are seen Anaphasic separation is normal and the two groups of chromosomes as soon as they reach the poles, organise themselves into interkinesis nuclei The nucleolus appears and the chromosomes which are more or less uniformly spaced are connected by thin strands (Fig 21) In Fig 22 is shown the second metaphase polar view There are two groups of nine univalents each Fig 23 shows the side view of the second metaphase The tetrads are formed by cell plate formation They may be either iso-bilateral (Fig 25) or tetrahedral (Fig 24) At the time of shedding, the pollen grains are two-celled (Fig 26) There are three germ pores

(c) *Megasporogenesis* —The ovary of *Vahlia viscosa* is bi-carpellary and uni-locular and the two parietal placentæ are pendulous and hang into the ovarian cavity (Fig. 5) The numerous anatropous ovules are arranged all around the placentas At a very early stage, a hypodermal archesporial cell is differentiated (Fig 27) Sometimes a plate of two archesporial cells is also found (Figs. 28 & 29) They may be either one below the other (Fig 29) or side by side (Fig 28) Multicellular archesporia are not uncommon in the Saxifragaceæ Pace (1912) in *Parnassia palustris* reports the presence of archesporial plates composed of two, three or four hypodermal cells The four cells were arranged in a linear row Multicellular hypodermal archesporia have also been reported in *Jamesia americana* (Mauritzon, 1933) No wall cell is cut off by the archesporial cell It begins to increase in size and functions directly as the megaspore mother cell (Fig. 30) Thus the genus *Vahlia* belongs to the " tenuinucellate "

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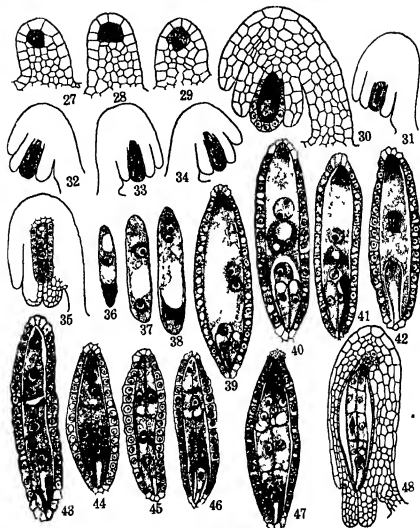
FIGS. 27-48   *Vahlia viscosa*, Roxb

Fig. 27 Hypodermal archesporium ×1500 Figs. 28 and 29 Two kinds of two-celled archesporial plate ×1500 Fig 30 Megaspore mother cell invested by the nucellus, ×1500 Fig. 31 Heterotypic division of the M M C ×350 Figs. 32-35 Various stages in the formation of the linear tetrad ×350 Figs 36-39 One, two, four, and eight-celled embryo-sac

In the 8-nucleate embryo-sac, the tapetal tissue does not surround the embryo-sac completely ×1500. Fig. 40 The polar nuclei are fusing, the synergida with the " synergidenhaken " Note the darkly-staining spherical body between the egg-cell and the polar nuclei ×1500 Fig 41 Fertilization The spherical body is present here also ×1500 Fig. 42 Division of the endosperm nucleus. ×1500 Fig 43 Two-celled endosperm Note the dark body in the micropylar endosperm cell and the persisting antipodals. ×1500 Fig 44 Longitudinal division of the micropylar endosperm nucleus In this case, the dark body is present in the chalazal chamber ×1200 Fig. 45 Four-celled endosperm ×1200 Fig 46 Transverse division of the two micropylar endosperm nuclei and the longitudinal division of one of the two chalazal endosperm nuclei ×1500 Fig 47 Shows four chalazal two middle and two micropylar endosperm cells ×1500 Fig. 48 Ovule showing later stage in the development of the endosperm and degenerating tapetum ×750

Saxifragaceæ In the " Krassu-nucellate " genera of the Saxifragaceæ, the archesporial cell cuts off a parietal cell which builds up a massive nucellus When there is a plate of two archesporial cells, one of them alone functions, for no case of two megaspore mother cells or two tetrads or two embryo-sacs was noticed in hundreds of sections examined Chapman (1933), however, found that the occurrence of two embryo-sacs in the same ovule was not rare in *Saxifraga virginiensis* In all these cases, the extra embryo-sacs were developed from a megaspore resulting from the division of a second megaspore mother cell In three out of five cases, each of the embryo-sacs was surrounded by its own nucellus, and in these, the embryo-sac was in the two or the four-nucleate condition In the other two cases, he found two megaspore mother cells, one of which was in the prophase of the first division, while in the other, the nucleus was in the metaphase of the first division In one case, the two megaspore mother cells lay side by side, while in the other, they were separated by two or three layers of nucellus This kind of archesporium which functions directly as the megaspore mother cell has been reported in *Jamesia americana, Philadelphus coronarius* (Mauritzon, 1933) and in *Parnassia palustris* (Pace, 1912) Just at the time, when the primary archesporium increases in size, to assume the functions of the megaspore mother cell, the primordia of the integuments arise The inner integument is the first to be initiated, while the outer soon follows the inner The inner integument is longer than the outer and it alone takes part in the formation of the micropyle (Fig 35) As the integuments grow, the ovule curves to assume its anatropous nature (Fig 30) At this stage, the megaspore mother cell could be seen to be invested almost to its base by a layer of cells, the nucellus (Fig 30) This nucellus is derived by repeated anticlinal divisions of the epidermal cells just above the primary archesporium In the Saxifragaceæ both single and two integumented ovules occur The genera *Ribes, Vahlia, Brexia, Parnassia,* etc , have two integuments, while the genera *Saxifraga, Kirengeshoma, Hydrangea,* etc , possess a single integument With the development of the megaspore mother cell, the cells of the

nucellus get flattened and finally disorganise and disappear completely, in the early stages of the development of the embryo-sac When the mega-spore mother cell has increased in size considerably, the heterotypic divi-sion sets in, as a result of which a dyad is formed (Fig 32) Fig 31 represents the telophase of the heterotypic division The cells of the dyad undergo the homotypic division to give rise to a linear tetrad (Fig 35) The dyads do not divide simultaneously Figs 32 to 34 show the various stages in the formation of the tetrad In Figs 32 and 33, the dyad cells are dividing simultaneously In Fig 32, the two nuclei are at metaphase In Fig 33, while the chalazal dyad is in the metaphase of the division, the micropylar dyad cell is in the telophase of the same division In Fig. 34, the chalazal dyad has divided to form two cells, while the micropylar cell is still in the anaphase of the first division Such a non-simultaneous division of the dyads has been recorded in *Parnassia palustris* (Pace, 1912) Here, while the chalazal dyad cell has only formed the chromosomes, the micropylar cell has already formed the spindle for the second division Though the linear arrangement of the tetrads is more common in the Saxifrageæ, T-shaped tetrads have also been reported in some cases Mauritzon (1933) reports the occurrence of T-shaped tetrads in *Bergenia crassifolia, Ribes aureum, Tiarella cordifolia, Heuchera sanguinea* and Chapman (1933) in *Saxi-fraga virginiensis* The chalazal megaspore is the functional one and the rest degenerate (Fig 36) The functional megaspore divides to give rise to the 2-nucleate embryo-sac (Fig 37) The four and the eight-nucleate embryo-sacs (Figs 38 & 39) are formed in the usual manner Fig 39 shows the mature embryo-sac, where the egg apparatus has been organised There are two prominent synergids, an egg cell, two polar nuclei situated usually in the middle of the embryo-sac and three antipodals towards the chalazal end The filiform apparatus described by Pace (1912) as being usual in *Saxifraga virginiensis* was not evident in the present case The " Synergidenhaken " however are seen, though not very markedly, as broad-ened bracket-like bases of the synergids (Fig 40) In the Saxifrageæ, these structures have been reported in *Hydrangea petiolaris, Francoa appendi-culata, Ribes stenocarpum, R nigrum, R grossularia, Mitella nuda* (Mauritzon, 1933), etc Fig 40 shows the two polar nuclei fusing in the centre The two nucleoli can still be seen The secondary nucleus is the largest and most prominent nucleus in the embryo-sac

The mature embryo-sac is roughly elliptical in shape The other genera of the Saxifrageæ have embryo-sacs of differing shapes (Mauritzon, 1933) The embryo-sac is long, narrow and cylindrical in *Francoa appendiculata* , highly enlarged in the micropylar region, while the chalazal portion is

narrow, wherein the antipodals are situated as in *Heuchera pubescens* and *Mitella nuda*, more or less elliptic as in *Itea virginica*, *Escallonia macrantha*, *Parnassia ovata*, the chalazal portion of the embryo-sac may be bent at an angle to the micropylar portion as in *Hydrangea petiolaris* In *Polyzoma ilicifolium*, the embryo-sac is obovate in shape, the chalazal end being broad and blunt, while in *Ribes missouriense*, the sides of the embryo-sac dilate and grow towards the chalazal end of the ovule like a haustorium

At about this stage, when the embryo-sac is fully organised and the polar nuclei have fused, a spherical, darkly-staining nucleus-like body makes its appearance just above the fusion nucleus This appears to be a feature of constant occurrence, for in about sixty or seventy embryo-sacs examined, we found this body always present It cannot, however, be a nucleus since there appears to be no definite nuclear membrane and also there is no nucleolus This body persists till after fertilization In Fig 41, where the remains of the pollen tube are seen and the egg cell is undergoing fertilization, we find this spherical body in a line with the fusion nucleus and the fertilized egg In Fig 43, where fertilization of the egg cell has been completed as could be seen from the degenerated synergids and where the endosperm has formed a 2-celled structure, we find this body again In Fig 44, which is a later stage showing 3-celled endosperm, we find this body below the chalazal endosperm nucleus Later than this stage we have not met with this body We are not at present able to offer any interpretation as to the exact nature of this spherical body

(d) Tapetum --As a result of the breakdown of the single layer of nucellus investing the megaspore mother cell, the innermost layer of the inner integument comes into direct contact with the sides of the embryo-sac This layer of cells, the tapetum, becomes conspicuous on account of the regularly arranged rectangular cells and they soon come to possess rich cell contents and hence stain rather darker than the other cells of the integument Often, however, small vacuoles could be noticed in the tapetal cells The cross walls are oblique The tapetum is thus of integumentary origin and its differentiation from the inner integument commences simultaneously from either end of the embryo-sac The tapetal cells are uni-nucleate though often bi-nucleolated (Fig 39) Bi-nucleate tapetal cells have been occasionally recorded in the Solanaceae (Bhaduri, 1932) and in the Orobanchaceae (Srivastava, 1939) The tapetum does not surround the entire embryo-sac At the chalazal and micropylar ends, only ordinary cells are found A distinct tapetal tissue surrounding the embryo-sac has been reported in various genera of the Scrophulariaceae (Srinivasan, 1940), Solanaceae (Bhaduri,

1935), Labiateæ (Billings, 1909), Lobeliaceæ (Kausik, 1938), Orobanchaceæ (Srivastava, 1939), Verbenaceæ (Tatachar, 1940), and in many other sympetalous families, as also in Crassulaceæ (Mauritzon, 1933) of the polypetalæ and in *Parnassia palustris* (Pace, 1912), *P ovata* and *Brexia madagascariensis* of the Saxifragaceæ (Mauritzon, 1933) In *Escallonia rubra*, and *Hydrangea petiolaris* also belonging to the Saxifragaceæ, the tapetal tissue is confined to the chalazal half, which is often bent at an angle to the micropylar half (Mauritzon, 1933) The function of the tapetum is essentially nutritive For, as the endosperm in the embryo-sac increases in size, the tapetal tissue gradually becomes thinner and thinner (Fig 48) and finally disappears.

(e) *Fertilization*—Fig 41 shows the male nucleus about to fertilize the egg The darkly staining pollen tube enters the embryo-sac through the micropyle The male cell would appear to be spherical Though vermiform and spiral-shaped male cells are by far the commonest in Angiosperms, spherical cells have however been occasionally reported, e g, Weinstein (1926) in *Phaseolus vulgaris*, Madge (1929) in *Viola odorata*, Newman (1934) in *Acacia Baileyana* Raghavan (1937) in *Cleome chelidonii*, and Raghavan and Srinivasan (1941) in *Ilysanthes parviflora* The male nucleus and the egg nucleus seem to be in a resting condition at the time of contact Such a condition is not only common in Angiosperms but also in the Coniferales (Guilliermond, 1933) and some Cycadales (Lawson, 1926) No phylogenetic significance can be attributed to this, as this phenomenon is found in such widely separated families as Oenotheraceæ (Ishikawa, 1918), Hydrocharitaceæ (Wylie, 1923), Orchidaceæ (Pace, 1907), Cappandaceæ (Raghavan, 1937), and Scrophulariaceæ (Raghavan and Srinivasan, V K , 1941) The synergids are ephemeral and degenerate soon after fertilization The antipodals often show a tendency to persist, though in a rather degenerated form, and are to be seen in embryo-sacs, in which the endosperm has become two to four-celled (Figs 43 and 46) In the Saxifragaceæ, antipodal haustorium is known to occur in *Kirengeshoma palmata* (Mauritzon, 1933), though all the three antipodals which are arranged one above the other in a linear fashion persist, the one towards the extreme chalazal end is elongated and is haustorial in its function

(f) *Endosperm*—The fusion endosperm nucleus is the largest nucleus in the post-fertilization embryo-sac. The position of the endosperm nucleus is always in the middle of the embryo-sac In the other genera of the Saxifragaceæ, the position of the endosperm nucleus varies considerably In *Mitella pentandra* (Dahlgren, 1930), the endosperm fusion nucleus is more towards the antipodal end The fusion nucleus undergoes a period of rest before it divides The first division is transverse and is accompanied by

wall formation. Fig 42 shows the fusion nucleus in the anaphase of the transverse division. The transverse wall divides the embryo-sac into two more or less equal halves (Fig 43). In the other genera of the Saxifragaceæ that exhibit cellular endosperm, the first wall is also transverse, though the two endosperm chambers that result in the embryo-sac are not equal as in the present case. Usually, in these, the chalazal endosperm chamber is considerably smaller than the micropylar one. Such a difference in size of the two cells of the two-celled endosperm stage has been figured for *Mitella pentandra*, *Boykinia occidentalis* (Dahlgren, 1930), *Boykinia Jamesii*, *Bergenia ligulata*, *Saxifraga micranthidifolia*, *Tiarella polyphylla* and *Ribes bureiense* (Mauritzon, 1933). Of the two endosperm cells thus formed, the micropylar one is the next to divide. This division is longitudinal followed by wall formation (Fig 44). Following closely on this, the chalazal cell also divides longitudinally (Fig 45). As a result of these two divisions, four endosperm cells are formed in the embryo-sac. This bears a close resemblance to the sequence of division of the endosperm nucleus in a few genera of the Scrophulariaceæ (Srinivasan, 1940). In the other genera of the Saxifragaceæ, however, the second and the third divisions are not longitudinal as in *Vahlia viscosa*. The second division is frequently transverse resulting in a row of three cells as in *Mitella pentandra* (Dahlgren, 1930). The two micropylar endosperm cells (Fig 46) then divide transversely followed by wall formation. As a result, six endosperm cells are formed in the embryo-sac. The two cells in the middle of the embryo-sac by repeated divisions form the cellular endosperm tissue. The two chalazal and micropylar cells also cut off cells towards the centre of the embryo-sac and contribute to the endosperm. The two chalazal cells often undergo a longitudinal division resulting in four cells arranged in two juxtaposed tiers one tier below the other (Fig 47). These cells become very rich in cytoplasm and hence stain brightly and the cells abutting on the chalazal end of the embryo-sac begin to exhibit signs of degeneration. These four chalazal endosperm cells thus appear to be haustorial in function, though they do not show any well-marked growth or haustorial protuberance penetrating the tissue around it. Though the endosperm cells towards the micropylar end also take a deep stain, they do not seem to be haustorial as the adjoining tissue does not show any signs of disintegration. As the endosperm tissue increases in size in the embryo-sac, the tapetal layer surrounding the embryo sac becomes thinner and thinner and in very late stages, it completely disappears. This is as it should be because the tapetum is essentially nutritive. As the endosperm develops rapidly, it absorbs nutrition required for its growth from the tapetum, which consequently becomes shrivelled up gradually.
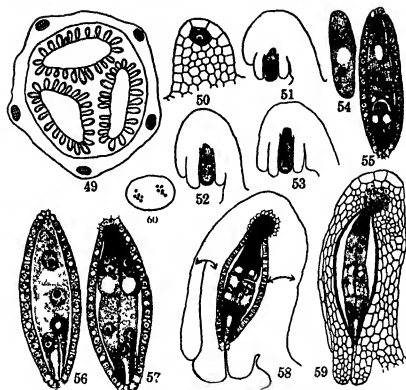
Figs 49-60   *Vahlia oldenlandioides*, Roxb

Fig 49   T S of ovary showing three placentas ×75   Fig 50   Hypodermal archesporium ×1500   Fig 51   M M cell with nucellus. ×350   Fig 52   Dyad in division showing six chromosomes ×350   Fig 53   Formation of the linear tetrad ×350   Fig 54   Two-nucleate embryo-sac   ×1500   Fig 55   Eight-nucleate embryo-sac, the polar nuclei fusing with the darkly staining body situated near it ×1200   Figs 56 and 57   Two- and four-celled endosperm ×1200   Figs 58 and 59   Ovules showing later stages of endosperm development ×750   Fig 60   Metaphase II, n = 6   ×2200

(g) *Embryo*—The oospore (Fig. 61) undergoes a long period of rest before it begins to develop into the embryo   The oospore divides long after the endosperm nucleus has divided   A stray case where in one embryo-sac, the oospore divides before the endosperm is reported by Pace in *Parnassia palustris* (1912)   Here, he observed a 5-celled embryo with the endosperm nucleus still undivided and somewhat amœboid in shape   After a considerable amount of endosperm tissue has been formed, during which

time, the oospore has elongated to some extent, it divides Consequently the oospore is surrounded by the endosperm tissue The first division of the oospore is transverse and is immediately followed by a cross-wall (Fig. 62) Of the two resulting cells, the lower one, *i e* , the one farther from the micropyle, gives rise to the embryo proper, and the upper, to the suspensor The lower or apical cell is the next to divide This division is anticlinal (Fig 63) Following closely upon this division, the upper one also divides, but transversely, both the divisions being followed by the formation of cross-walls (Fig 64) Within the Saxifragaceæ, a similar behaviour of the 2-celled embryo has been figured in *Parnassia palustris* (Pace, 1912) In this also, while the apical or lower cell divides longitudinally, the basal or upper cell divides transversely There is however some difference in the timing of the two divisions between the two-celled embryo in *Parnassia* and in the present case For, while in *Vahlia viscosa*, the lower (embryonal) cell divides before the basal cell, in *Parnassia palustris*, from the figure given by Pace (1912), it would appear that the upper cell begins to divide a little earlier than the apical cell In Fig 64, the lower cell has divided while the upper is in the late telophase of the transverse division As a result of these two divisions, a four-celled pro-embryo is formed, in which the two lower cells are placed side by side, while the upper two are placed one above the other (Fig 65) and as such the 4-celled pro-embryo is only three cells long This type of arrangement of the four cells of the pro-embryo is common in families like Cruciferæ, Ranunculaceæ (Souges, 1913, 1919), Capparidaceæ (Raghavan, 1937), etc In the other type, the four cells of the pro-embryo are arranged in a linear fashion Such an arrangement of the four cells of the pro-embryo is characteristic of the Rubiaceæ (Raghavan and Rangaswamy, 1941), Solanaceæ (Souges, 1922), Leguminoseæ (Cooper, 1933), Orobanchaceæ (Srivastava, 1939), Scrophulariaceæ (Srinivasan, 1940), and in the Acanthaceæ (Rangaswamy, 1941) In the Saxifragaceæ, both types of arrangement of the four cells in the pro-embryo are met with For instance, in *Ribes divaricatum*, in *Astilboides tabularis* and in *Heuchera sanguinea* (Mauritzon, 1933), the four-celled pro-embryo is linear, while in *Parnassia palustris* (Pace, 1912), the arrangement of the four-celled embryo is similar to that found in the present investigation Besides these two types of arrangement, often a linear pro-embryo of five or more cells in length is also to be found as in *Boykinia tellimoides* (Dahlgren, 1930), and *Tiarella polyphylla* (Mauritzon, 1933) The four-celled embryo stage is an important one, as each one of these four cells gives rise to a definite region in the mature embryo For purposes of description, these four cells beginning from the uppermost or basal cell will be designated A, B, C and D The cell B is the

first to divide among the four cells of the pro-embryo It divides by a transverse wall (Fig. 66) into B_1 and B_2, as a result of which the suspensor becomes three cells long (Fig 67) Often this division is followed by an oblique cross-wall as shown in Fig 68 The suspensor becomes four cells long
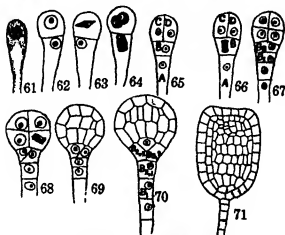
Figs 61-71 *Vahlia viscosa*, Roxb

Various stages in the development of embryo　Figs 61-70 ×1500　Fig. 71 ×1100

by another transverse division of the cell A　The cell B_2 divides transversely into two cells B_{2+1} and B_{2+2} of which the latter is the hypophysis. The cell B_{2+2} cuts off a cell which abuts into the embryonal sphere and is itself divided into two cells by a vertical wall (Fig 70)　Figs 69 and 70 are those of later stages in the embryo development and show the differentiation of the primary tissues, dermatogen, periblem and plerome　In the mature embryo, the suspensor is about 5 to 6 cells long and is uni-seriate (Fig. 71)　Multiseriate and massive suspensors especially at the base, are a common feature of most members of the Saxifragaceæ　Multi-seriate suspensors have been recorded in *Mitella dyphylla*, *Sullivantia sullivantii*, etc., and in *Ribes aureum*, the suspensor is very massive and irregular in shape

IV　*Vahlia oldenlandioides*, Roxb

The two species of *Vahlia* investigated here, are characterised by parietal placentation　The placentas are pendulous and hang from the roof of the gynæceum into the ovarian cavity as has already been described.　In *V. oldenlandioides*, however, often three parietal placentas and three styles

are to be found, and Fig 49 shows the transverse section of such an ovary The development of the ovule, embryo-sac, the endosperm and of the embryo bears a striking resemblance to that already described for *V viscosa* The primary archesporium which is hypodermal may consist of a single cell (Fig. 50) or a plate of two cells This species of *Vahlia* is also characterised by the possession of two integuments, the inner of which alone takes part in the organisation of the micropyle The nucellus is also similar to that in *Vahlia viscosa* Fig 52 represents the dyad The nuclei of the dyad are in the metaphase of the homotypic division and in the miropylar dyad, the haploid chromosome number of six is clearly seen A linear tetrad is formed (Fig 53), the chalazal one of which develops into the mature embryo-sac in the usual manner (Fig 55) Fig 54 shows the bi-nucleate embryo-sac The mature embryo-sac bears a close resemblance to that of *V viscosa* and is surrounded by a tapetum of integumentary origin The tapetal cells are uni-nucleate Even in this species the darkly staining spherical body described for the other species was found to be a feature of constant occurrence. The endosperm cells towards the chalazal end have a haustorial function as in the other species already described

Thus we find that the life-histories of the two species of *Vahlia* are very similar and it would appear that the genus *Vahlia* is characterised by the possession of two or three parietal and pendulous placentas, ovules with two integuments and a single layer of nucellus which degenerates very soon giving place to a tapetal layer of integumentary origin, the tapetal cells being uni-nucleate The chalazal megaspore of a linear tetrad always forms the embryo-sac, which is normal, the degenerated antipodals often persisting till the two-celled endosperm stage Endosperm is cellular, and in both the species, the plan of cellular endosperm development is different from the other genera of the Saxifragaceæ so far studied, but is similar among themselves In the four-celled pro-embryo, the two apical cells are placed side by side while the two basal cells are one over the other and the suspensor is uni-seriate and about 5 cells long

The haploid chromosome number has been determined to be 6 and this finding corroborates the number already recorded by Skovsted (1934) Fig 60 is that of M II in polar view and shows two groups of six univalents each

V Discussion

(a) *The Endosperm* —The family Saxifragaceæ is interesting especially from the point of view of endosperm development Within the family, three distinct types of endosperm development are found. Some genera are characterised by the cellular type , in some others the free

nuclear type of endosperm development is the rule The third or the "Helobiale type" is a combination of the cellular and the free-nuclear type of endosperm development In this last mentioned type the first one or two divisions are followed by the formation of cross-walls while successive divisions produce free nuclei, wall formation commencing later Of the two cells resulting from the first division, in one the development of the endosperm will be cellular, while in the other cell it is exclusively free-nuclear Mauritzon (1933) has classified the plants investigated till his time into three groups on the basis of the nature of endosperm development According to him, the genera *Brexia, Parnassia, Tetilla* and *Francoa* have the "nuclear type" of endosperm The genera *Astilbe, Astilboides, Bergenia, Boykinia, Cardiandra, Decumaria, Deutzia, Heuchera, Hydrangea, Jamesia, Kirengeshoma, Lithophragma, Mitella, Peltiphyllum, Philadelphus, Ribes, Rodgersia, Tellima, Tiarella, Tolmiea* and *Vahlia* exhibit the "cellular type" of endosperm development, while the genera *Boykinia, Chrysosplenium, Mitella, Ribes, Saxifraga, Sullivantia* and *Tiarella* possess the "Helobiale type" of endosperm in which endosperm development is cellular to begin with but later becomes free-nuclear There are, as will be seen from the examples cited, a number of cases, in which the two types of endosperm development are found in the same genus For example, in the genus *Mitella,* the species *M diphylla* exhibits the "Helobiale type" of endosperm development, while in *M pentandra* its development is exclusively "cellular" Again in *Boykinia, B aconitifolia, B Jamesii* and *B occidentalis,* show the cellular type of endosperm, *B tellimoides,* however, is characterised by the "Helobiale type" of endosperm Similarly in the genus *Tiarella* while some species show the "Helobiale type" of endosperm, a few other species exhibit "cellular" type of endosperm Thus we find that in the Saxifragaceæ endosperm development varies considerably from genus to genus and even within the same genus, different species exhibit different type of endosperm development In the two species of *Vahlia* investigated, cellular endosperm is the rule Taking into consideration the cellular type of endosperm development in the Saxifragaceæ, we find that all the species showing cellular endosperm do not follow the same plan or sequence of divisions in the building up of the endosperm Even here, we find that different genera show different plan or sequence of divisions in the formation of the endosperm tissue In the Saxifragaceæ, among those exhibiting cellular endosperm the more usual method seems to be the formation of three endosperm cells by two transverse walls laid across the embryo-sac, and during the further development of the endosperm tissue a longitudinal wall is usually laid in the chalazal chamber to begin with,

and similar longitudinal walls are formed in the other two endosperm cells also This type of cellular endosperm development has been noted by Mauritzon (1933) in *Astilboides tabularis, Mitella nuda, Bergenia ligulata,* etc , while in others like *Boykinia occidentalis* (Dahlgren, 1930), the second division takes place in the chalazal cell and is longitudinal In the present investigation, the two species of *Vahlia* studied present an altogether different plan of endosperm development from the others The first division is transverse, while the second division is longitudinal and takes place in the micropylar endosperm cell The third division is also longitudinal and takes place in the chalazal cell The two micropylar cells then divide transversely Such a plan of division of the endosperm nucleus has not so far been reported in any other member of the Saxifragaceæ A similar scheme of endosperm development has been found in *Dopatrium lobelioides, Stemodia viscosa* and *Vandellia crustacea* all belonging to the Scrophulariaceæ (Srinivasan, 1940)

While in the closely related family of Crassulaceæ (Mauritzon, 1933) haustoria of diverse origin, like megaspore haustorium, suspensor haustorium, chalazal and micropylar endosperm haustorium are common, in the Saxifragaceæ, however haustoria are of rare occurrence In *Kirengeshoma palmata* (Mauritzon, 1933) antipodal haustoria are present Micropylar haustorium of endospermal origin has been recorded by Mauritzon (1933) in *Corokia cotoneaster,* in which is figured a single uni-nucleate micropylar endosperm haustorium Four uni-nucleate vermiform micropylar haustoria occur in *Philadelphus coronarius* (Mauritzon, 1933) The function of these haustoria is the usual one of supplying nutrition to the developing endosperm and embryo

An effort was made to find out whether a correlation could be established between the division of the family on the basis of the type of endosperm development and the division of the family by taxonomists on morphological grounds We also endeavoured to discover whether chromosome numbers that are known so far in this family could, in any way be employed for the elucidation of this In this connection, we gathered most of the available data regarding this family, both morphological and cytological Engler recognises seven sub-families and in trying to find out if a relationship could be established between the type of endosperm formation as available from previous work and this classification, we found that within each sub-family there occurred almost all the three types of endosperm development In some cases, as in the genus *Ribes,* even within a genus, different types of endosperm development are reported The statement given below will show this point clearly For instance, in the sub-family Saxifragoidæ, *Vahlia* shows the "cellular type," so also *Astilbe, Parnassia,* shows "nuclear",

Chrysosplenium and *Saxifraga* show the "helobiale type" of endosperm development The Hydrangeoideæ is a bit more uniform where the three genera about which information is availabe show the "cellular type" of endosperm but no generalisation is possible since we have no information about the other sixteen or seventeen genera comprised in this sub-family In the tabular statement presented, only important genera included in the sub-families are shown, it will be seen that practically no information is availabe about the method of endosperm formation in the sub-families Petrostemonoideæ, Escallonoideæ, and Baueroideæ But the information available at hand shows clearly that there could be no question of the division of the family on the basis of the method of endosperm formation The chromosome numbers of a comparatively large number of genera included in this family are known But these are confined principally to the sub-families Saxifragoideæ, Hydrangeoideæ and Ribesioideæ Some of the numbers known are noted against the respective genera It could be seen that aneuploidy has played, presumably, a very important part in the evolution of the genera and that no support could be had from these numbers for tackling the problem cyto-taxonomically We also endeavoured to see if the chromosome numbers and the type of endosperm development could in any way be related For example, the species showing the "cellular type" of endosperm exhibit numbers 6, 7, 8, 9, 13, 16, 18, 38, 39, 52, etc Similarly, the "nuclear" forms exhibit 9, 10, 20 and those belonging to the "Helobiale type" show also a similar range of chromosome numbers 8, 9, 11, 13, 16, 18, 20, 24, etc It is obvious from these data that have been presented, that it may not be easy to classify the family easily, from these points of view It has already been said that it is a rather heterogeneous family , the position of some members is even doubtful, as for example that of *Parnassia* already mentioned If the problem is to be tackled cyto-taxonomically, it would be better to arrange the different genera and species according to the respective chromosomal series those belonging to the 8 series, 9 series and so on The genus *Deutzia* for instance would appear to fall in with the 13 series, so also *Philadelphus Hydrangea* belongs to the 9 series In *Saxifraga* we get an aneuploid series 8, 9, 11, 13, 18, and so on Very likely, a more critical cytological examination may throw some light upon the basic number of the genus and also that of the sub-family In this, phenomenon like secondary association coupled with some genetical data will undoubtedly play a very large part It may be that there is a primary basic number from which there might have arisen other secondary basic numbers, each of which might have produced their own polyploid series How these secondary polyploid series arose can again be inferred only by a cytological and genetical study In this

way, if data are gathered, it may be possible to tackle the problem cyto-taxonomically   Pending this, it can only be said that the family is indeed, in the words of C B Clarke (1879) " very difficult of definition "

I   *Saxifrageæ*

    H   Saxifraga (6, 7, 8, 9, 13, 14, 15, 16, 18, 36, 39, 52, 65)

    Z   Vahlia (6, 9)

    H   Chrysosplenium (12, 24)

    Z   Heuchera (7, 8)

    N   Parnassia (9, 10)

    Z   Astilbe (7)

    Z & H   Tiarella (9)

II   *Francooeæ*

    N   Francoa (20)

    N   Tetilla

III   *Hydrangeoideæ*

    Z   Hydrangea (18, 36)

    Z   Philadelphus (13)

    Z   Deutzia (13, 39, 52, 65)

IV   *Pterostemonoideæ*

    Pterostemon

V   *Escallonoideæ*

    Escallonia

    Phylloma.

    Polysoma

    Itea

VI   *Ribesiodeæ*

    Z & H   Ribes (8, 16)

VII   *Baueroideæ*

    Bauera

*N B —*The numbers given in brackets on the right-hand side of the genera are the chromosome numbers prevalent in the respective genera, and the bold letters on the left-hand side denote the type of endosperm development found in the respective genera

        Z = Cellular endosperm

        N = Nuclear endosperm.

        H = Helobiale type of endosperm

(b) *The Nucellus and the Tapetum* —The family Saxifragaceæ is interesting not only from the point of view of endosperm formation but also from that of presence of the tapetum   In this connection, we have tried to gather available information regarding the tapetum, endosperm formation and the

nature of the nucellus and the possible correlation between these. Generally speaking, nuclear type of endosperm formation is associated with a massive nucellus. This is widely prevalent amongst the apetalæ and polypetalæ of Bentham and Hooker. In these, there is no tapetum formation. This statement does not mean, however, that this is a rigid rule. There are some families amongst this group of Angiosperms where we get reduced nucellus "Tenuinucellate" (single layer of nucellus) as for instance in Sarraceniaceæ, Podostomaceæ, Pittosporaceæ, etc. There are also a few cases in this group, where the massive nucellate condition "Krassinucellate" is associated with cellular endosperm. All that the statement implies is that in the vast majority of the families comprised in this group, massive nucellus and nuclear endosperm coupled with the absence of any tapetum seems to be the rule. In the Sympetalæ, etc. the reduced nucellus is the prevailing condition. As in the previous case, there are found a few families in which this reduced nucellate condition (tenuinucellate) is associated with nuclear endosperm for example, Gentianaceæ, Apocynaceæ, Loganiaceæ, Asclepiadaceæ, Rubiaceæ, Goodeniaceæ, etc. It is amongst this group, that we get the tapetum and it is in some of the families comprised in this group that the endosperm haustorium is prevalent. The pertinent question arises, whether there is any factor which governs the appearance of the tapetum. The tapetum such as occurs in the ovule, is almost always integumentary in origin, and must be regarded as nutritive in function even as the microsporangial tapetum. While there can be no doubt as to the nutritive character of the latter, because of its universal occurrence, the same cannot be said of the integumentary tapetum, because it is confined only to some families. In order, therefore, to find out its true role, and also if possible, the conditions under which it usually occurs, we tried to see if a correlation could be discovered between this and the other associated tissues like the nucellus and the endosperm. Such an investigation has revealed as has been indicated, the existence of some relationship from which could be drawn a few inferences which for the moment must obviously be regarded as tentative. The first observation of importance is that the tapetum almost always is integumentary in origin. That is, it occurs only where there is no parietal tissue. A possible inference from this is that where there is no massive (Krassi-nucellus) parietal tissue, the nutrition of the embryo-sac is presumably defective, however much its place may be taken up by the integument. Naturally, in order to strengthen the nutritive mechanism, the tapetal layer is present. Support to this can be gathered from the fact that the occurence of the tapetum is mostly associated with cellular endosperm, many of which exhibit some type of haustorium or other. This means that because the

nutritive mechanism is not perfect, recourse has been taken to these supple-
mentary devices, by which to make up for the deficiency The next question
is in what way does the tapetum discharge its nutritive role? If an analogy
is to be established between this tapetum and the anther tapetum we must
naturally look for the pluri-nucleate condition of the tapetal cells and the
subsequent usage of this nuclear material for the nutrition of the embryo-sac
Though this multi-nucleate condition is widely prevalent so far as the anther-sac
tapetum is concerned, this appears to be rather the exception than the rule so
far as the integumentary tapetum is concerned This leads to the question
whether the tapetal cells directly contribute to the nutrition of the embryo
sac or does the tapetal layer merely act as a sort of a liaison tissue, merely
helping in the transference of nutritive material from the surrounding integu-
mentary tissue To our mind the latter alternative seems to be the more
possible, for as has already been said, this tapetum occurs only where the
massive (Krassi) nucellate condition does not exist In this "Krassi-
nucellate" condition, the embryo-sac is closely surrounded by the parietal
tissue and naturally there can be no difficulty whatsoever in the matter of
the supply of food material by this closely enveloping tissue to the embryo-
sac But in the "tenuinucellate" condition the embryo-sac is left severely
alone The single layer of nucellus very soon perishes in the ontogeny of the
ovule There is a gap between the developing embryo-sac and the developing
integument There can be no question of a close contact of these two, such
as exists between the parietal tissue and the embryo-sac in the Krassi-nucellate
condition Naturally, when contact, however imperfect is established,
between the developing embryo-sac and the integument some sort of an
intermediary tissue is found necessary in order to facilitate the free transference
of nutritive material from the integument to the embryo-sac Presumably,
even this device is not sufficient for in many cases the endosperm haustorium
makes its appearance as a post-fertilization structure The tapetum after
performing its function disintegrates, when the endosperm tissue is begin-
ning to take up the nutritive role

VI *Summary*

The haploid chromosome number of *Vahlia oldenlandioides* has been
confirmed to be 6, and that of *V viscosa* has been determined to be 9

The origin and development of the microsporangium, the embryo-sac,
the endosperm and the embryo is described in detail, both *Vahlia viscosa*
and *V oldenlandioides* are found to be quite similar

In both the species of *Vahlia* investigated, the endosperm is cellular and
there are four uninucleate chalazal endosperm haustorial cells in both

No relationship could be established between the division of the family on the basis of the type of endosperm development with (i) division of the family by taxonomists on morphological grounds and (ii) the chromosome numbers known in the family

For a cytotaxonomical approach, cytological details like secondary association, etc of which no information is now available, are suggested to be a necessary prerequisite

The role of the integumentary tapetum is discussed in the light of its correlation to the nucellus and the endosperm

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TRYPSIN-KINASE IN BLOOD

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SCHMITZ[1] has isolated a trypsin-inhibitor from beef blood and has shown that it is probably a polypeptide of low molecular weight This inhibitor exists in combination with trypsin in the circulating blood, thus inactivating most of the enzyme The author makes the statement that this trypsin-inhibitor is present in blood in excess of the amount required for the complete inactivation of trypsin present in blood If this were the case the acetone precipitate of the plasma should not possess any proteolytic activity Iyengar[2] and Scott have reported a definite though small, tryptic activity in the plasma proteins obtained by precipitation with acetone This however does not disprove the existence of the inhibitor, the presence of which has been substantially confirmed by us in our experiments on the proteolytic activity of various blood fractions Trypsin circulating in plasma is only partially neutralised by the inhibitor and a small portion of the trypsin is left free to exert its activity The significance of this free plasma trypsin in physiological and pathological conditions, is under investigation

The proteolytic system in blood has been the subject of extensive investigations by Schmitz, who has experimentally demonstrated a combination of trypsin with inhibitor to form a trypsin-inhibitor compound which as such is inert The enzyme is liberated in an active form by treatment with trichlor-acetic acid bringing the pH of the plasma to 3 and breaking the compound into its constituents If the plasma-trypsin is to play a role in the physiological processes, there must be a mechanism in the body to bring about such a breakdown of the trypsin-inhibitor compound The pH of the blood *in vivo* is at no time as low as 3 and hence the mechanism of activation of plasma-trypsin *in vivo* if present must be other than by acidification Schmitz envisages the possibility of the presence in blood of a ' kinase ' similar to the trypsin-kinase present in the intestinal secretions bringing about the activation of trypsinogen of the pancreas No direct evidence has been adduced to support the above hypothesis According to this author,

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the ' kinase ' also combines with the trypsin-inhibitor present in the blood, and when present in such a combination, it is not capable of liberating the trypsin from the trypsin-inhibitor compound Summing up the above observations, it is seen that there is present in plasma a trypsin-inhibitor compound and a kinase-inhibitor compound both of which are unable to exert their respective actions It is presumed that the inhibitor is the same chemically in both the compounds

Pope[3] during the course of his work on the purification and concentration of diptheria anti-toxin, has detected the presence of a proteolytic enzyme in fibrin clots Since this enzyme lyses the fibrin Pope named it fibrinolysin The enzyme fibrinolysin is also reported to be present in the culture medium in which *Streptococcus* is grown Judging from the properties of this enzyme, I am of opinion that the nomenclature, fibrinolysin, can be employed only to the enzyme which digests specifically the fibrin protein, and does not attack other proteins The proteolytic enzyme present in the fibrin clot appears to resemble trypsin in its pH optimum and in its action on other proteins Trypsin it may be stated, can also digest the fibrin clot I am of opinion therefore, that the so-called fibrinolysin reported by Pope is nothing but plasma-trypsin in its free and active state

Schmitz has put forward a very interesting hypothesis to explain the presence of trypsin in the fibrin clot

According to Schmitz, during clotting, the trypsin-inhibitor compound is adsorbed on the fibrin clot The kinase-inhibitor compound which is also present in plasma, is broken up into its constituents with the result that only kinase which is probably of a protein nature gets adsorbed on the clot while the inhibitor which is a polypeptide of low molecular weight remains in the serum The components of the proteolytic system present in the fibrin clot are (i) trypsin-inhibitor compound and (ii) trypsin-kinase Under these circumstances, it is reasonable to visualise an interaction of the two components if the fibrin is suspended in a buffer suitable for kinase action This reaction can be represented by the following equation

Trypsin-inhibitor compound + Kinase ⇌ trypsin + Kinase-inhibitor

It can be seen from this equation that trypsin is liberated in an active form during the process of clotting by the mechanism described above This fascinating possibility is based on the assumption that the trypsin-kinase is present in the blood, for which no direct evidence has been presented as yet As I was engaged in a fairly detailed study of the proteolytic system present in the whole blood, it occurred to me that it might be worth while to

investigate the various blood fractions for the presence of this kinase
In pursuance of this idea, the following constituents of blood have been
examined

    (i) Red Blood Corpuscles
    (ii) Platelets

Experimental

(1) Red blood cells from horse were laked with distilled water and precipitated with 4 volumes of acetone at a temperature of 10° C , centrifuged and washed with acetone

(2) Citrated horse blood was kept in the refrigerator and red blood cells were allowed to settle The plasma was then taken off and centrifuged at the rate of 3,000 revolutions per minute The sediment was taken in a jar and allowed to settle in the refrigerator The supernatant was siphoned off and the sediment resuspended in citrates, this suspension was precipitated with 4 volumes of acetone in the cold, centrifuged and the precipitate again washed with 1 acetone The precipitate was finally dried at room temperature This is the platelet preparation

(3) *Trypsin-inhibitor Compound* —This is present in plasma as a protein and hence is precipitated by acetone 100 c c of citrated plasma were precipitated by the addition of 400 c c of acetone The precipitate was first washed with aqueous 90% acetone, then with pure acetone and finally dried at room temperature This preparation has been shown by Iyengar and Scott, to possess slight proteolytic activity

(4) *Trypsin from Plasma freed from the Inhibitor* —100 c c of citrated plasma were precipitated with 11 volumes of 2 5% trichlor-acetic acid The precipitate was centrifuged thoroughly freed from the residual nitrogen by washing with 2 5% trichlor acetic acid The still moist precipitate is dissolved in 500 c c of water and this solution was mixed with two litres of acetone Upon the addition of a small amount of sodium acetate solution, the plasma was again precipitated and now washed with acetone This precipitate was dried at room temperature This preparation has a greater proteolytic activity than preparation 3, since the trypsin-inhibitor compound has been broken up by treatment with trichlor-acetic acid

1 gm. of each of the above preparations were suspended in 20 c c of M/15 phosphate buffer of pH 8 4 and incubated for 18 hours at 37° 5 C The increase in non-protein nitrogen in each case was as follows —

TABLE I

Red Blood Cells	Platelets	Trypsin-inhibitor Compound	Plasma-Trypsin freed from the Inhibitor
mg	mg	mg.	mg
2 2	9 48	1 57	3 51

If the kinase is present in red blood cells, it should be able to liberate the trypsin from the trypsin-inhibitor compound when incubated with the latter in M/15 phosphate buffer of pH 8 4 The following experiments were carried out to ascertain the presence of kinase in red blood cells

1 gm of the red cells was mixed with 1gm of the trypsin-inhibitor compound preparation, the mixture suspended in 20 c c of M/15 phosphate buffer of pH 8 4 and incubated for 18 hours at 37° 5 C In another experiment 1 gm of red blood cells was mixed with 1 gm of the plasma-trypsin preparation freed from the inhibitor, the mixture also suspended in 20 c c of M/15 phosphate buffer of pH 8 4 and incubated for 18 hours at 37° 5 C The increase in non-protein nitrogen in each case was as follows —

TABLE II

		Increase in N P N
		mg
1	Red Blood Cells	2·2
2	Trypsin-inhibitor Compound	1 57
3	Red Blood Cells and Trypsin-inhibitor Compound preparation	3 9
4	Plasma-Trypsin preparation freed from the inhibitor	3 51
5	Red Blood Cells *plus* Plasma-Trypsin freed from the inhibitor	5 95

If kinase was present in red blood cells, the non-protein nitrogen in the case of (3) should be approximately the same as in (5), since the latter represents the combined proteolytic activity of red blood cells and the plasma-trypsin freed from the inhibitor by treatment with trichlor-acetic acid The increase in non-protein nitrogen in (3) is 3 9 mg which is approximately equal to the increase in N P N of (1) and (2) added together The above results definitely show that trypsin-kinase is not present in red blood cells

Attention was then directed to the platelet preparation Similar experiments as in Table II were conducted with the only difference that in place of

red blood cells, the platelet preparation was employed The results of
these experiments are.—

TABLE III

		Increase in N P N mg
1	Platelet preparation	9 48
2	Trypsin-inhibitor compound preparation	1 57
3	Platelet preparation *plus* Trypsin-inhibitor compound preparation	13 05
4	Plasma-Trypsin preparation freed from the inhibitor	3 51
5	Platelet preparation *plus* Plasma-Trypsin preparation freed from the inhibitor	13 65

The results reported above lend direct experimental evidence for the
presence of trypsin-kinase in platelets The proteolytic activity of (3) is the
same as (5) within the limits of experimental error If kinase was not
present in the platelets, the proteolytic activity of (3) should have been
represented by an N P N increase of (1) + (2) (i.e., 9 48 + 1 57 = 11 05
mg) whereas the actual increase is 13 05 mg The increased tryptic
activity is due to the extra amount of trypsin released from the associate
inhibitor by the kinase that might be present in platelets The fact that the
proteolytic activity of (3) is approximately the same as in (5), further shows
that the trypsin-inhibitor compound is practically completely broken up by
the platelets

It may be argued that the increased tryptic activity of (3) may be due to
the addition of an increased amount of protein, since the trypsin-inhibitor
compound added to the platelet preparation, is merely plasma proteins
directly precipitated by acetone Such a possibility does not however exist,
because the platelet preparation itself contains a very large proportion of
substrate compared to the quantity of associated trypsin, and the percentage
protein broken up by auto-digestion is only 6% Besides, the enzyme being
simultaneously precipitated with the platelet proteins by acetone, the
trypsin is already adsorbed on the platelet proteins and will therefore have a
preference to digest the associated protein which is present in plenty beyond
the capacity of the enzyme present to digest. Iyengar (*I J M R*, July 1941)
has shown that, if to an enzyme preparation containing a large quantity of a
susceptible protein substrate another protein which is less susceptible is
added, the latter remains practically unattacked When insulin was added
to a platelet preparation (obtained by acetone precipitation) and incubated
for a period of 24 hours, the hormone remained practically intact which was
demonstrated by the complete retention of its physiological activity. The

plasma proteins obtained by acetone precipitation if incubated in M/15 phosphate buffer undergoes auto-digestion to the extent of only 1% So, in this case also, the associated trypsin has a plentiful supply of substrate already and the addition of the platelet preparation should not make any difference so far as the tryptic activity of the plasma proteins are concerned Therefore if the tryptic activity of the mixture of platelets and plasma proteins (obtained by acetone precipitation) is significantly more than the added value, the only possibility is that some interaction between the two has taken place giving effect to this increased activity This interaction is between kinase probably present in platelets and the trypsin-inhibitor compound known to be present in acetone precipitated plasma proteins as hypothetically visualised by Schmitz It may be stated that the above results may not be direct convincing evidence for the presence of kinase in platelets, since the kinase has not been isolated from the platelet-proteins and the associated trypsin, but the results reported in this paper lend strong experimental support for the presence of kinase in blood

Summary

A clear picture of the proteolytic system existing in blood has been presented

The possibility of the presence of a trypsin-kinase in blood has been discussed

The red blood cells and the platelets have been examined for the presence of trypsin-kinase

Red blood cells do not contain the kinase

The experiments reported in this paper strongly suggest the presence of trypsin-kinase in platelets, which is capable of liberating the trypsin from the inhibitor compound present in acetone precipitated plasma proteins

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ANTI TRYPTIC COMPONENTS OF BLOOD

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It is well known that the blood serum has the property of retarding the digestive activity of trypsin Landsteiner[1] reported that this effect was associated with the albumin fraction of serum Hedin[2, 3] found many similarities between the inhibition of trypsin by adsorption on charcoal and inhibition of trypsin by serum albumin and therefore came to the conclusion that the equilibrium between trypsin and serum albumin was governed by adsorption phenomena, *i e*, the trypsin is adsorbed by the serum albumin as it is by the charcoal The correctness of this interpretation is questioned by Hussey and Northrop[4] after a detailed study of the mechanism They have adduced evidence which suggests that the inhibitive agent in serum combines with trypsin to form an inactive but dissociable compound The conditions of equilibrium are apparently governed by the law of Mass-Action

It has been shown by Schmitz[5] that the enzyme trypsin present in plasma is blocked by an inhibition body, whose action is in no way related to the anti-tryptic action of serum albumins This inhibitor could be separated from the serum albumins by precipitation of proteins with trichlor-acetic acid whereby the inhibitor remains in solution This can also be prepared by ultra-filtration of an acid solution of plasma proteins obtained by acetone precipitation By this process, the inhibitor passes into the albumin-free filtrate This inhibitor is thus strongly analogous to the trypsin-inhibitor obtained by Kunitz and Northrop[6] from the pancreas The reaction between Schmitz plasma-inhibitor and crystalline trypsin shows that it runs quantitatively as in the case of Northrop-inhibitor from the pancreas The one important difference between the two inhibitors is that plasma-inhibitor does not inactivate chymo-trypsin from the pancreas as opposed to the pancreas-inhibitor which reacts with chymo-trypsin bringing about a gradual fall in activity By this process, the inhibitor associated with the serum albumin also inactivates chymo-trypsin and hence is similar only in this property to the inhibitor from pancreas The inhibitor present in native serum is however differentiated from either the inhibitor from pancreas or the inhibitor isolated from plasma by Schmitz According to Hedin[7] the inhibition of trypsin appears only when ferment and serum are mixed first and after an interval the substrate is added On the other hand, the other two inhibitors

act only if they are added to the ferment substrate mixture when the digestion is in progress The destruction of trypsin by serum (albumin) is therefore sharply differentiated from the inhibition by the substance isolated from blood plasma In blood plasma, there are therefore two inhibition mechanisms side by side, of which one is probably (according to Schmitz) by unspecific adsorption of the ferment on serum albumin and difficulty of elution and the other by a quantitative combination with the ferment specifically In order to detect the presence of the second inhibitor, either free inhibitor should be present in plasma or it should be separated from the trypsininhibitor compound

During the course of a search for a simple substance which might retard the activity of specific proteases, it was discovered by Horwitt[2] that heparin, the physiological anticoagulant is a trypsin-inhibitor The effects of heparin on the hydrolysis of casein by crystalline trypsin and chymo-trypsin isolated by beef pancreas, have been studied No inhibition of trypsin is obtained unless the heparin is allowed to remain in contact with the alkaline trypsin solution for about 30 minutes before the two are added to casein This inhibition of trypsin by heparin has many similarities with the mechanism of inhibition of trypsin by the inhibitor isolated from plasma The heparin-trypsin addition complex like the trypsin-inhibitor compound may be dissociated by acidifying to pH 3 for about 30 minutes to recover all the tryptic activity Like the plasma-inhibitor, heparin does not inhibit chymotrypsin This property of heparin is very interesting since trypsin catalyses the clotting of recalcified plasma while chymotrypsin does not It has been reported by Horwitt that trypsin and heparin are mutually antagonistic in a sample of plasma and that clotting will not occur unless the amount of trypsin added is more than enough to neutralise the effect of heparin

It is evident from the foregoing account that there are present in blood, three different substances having the property of retarding the activity of trypsin The presence of these substances acquires an added significance in the light of my (Iyengar, N K)[3] observations on the presence of trypsin in plasma and its possible role as the physiological thrombo-plastic substance

The part played by trypsin in blood in the inactivation of insulin has been discussed by Iyengar and Scott [10] In view of this dual role of trypsin in blood, the action of the anti-tryptic components of blood in retarding coagulation or in prolonging the hypoglycemic effect of insulin is worth investigating The object of this paper is to study first of all the *in vitro* effect of these trypsin-inhibitors on the action of trypsin in (i) catalysing the coagulation of blood and (ii) in the destruction of insulin The action of heparin as a general anti-coagulant is well known and therefore need not

be considered The action of heparin in checking the destruction of insulin by trypsin has not been reported, although the possibility is indicated by Horwitt (Science, 1940) Long before Horwitt's work was published, while I was working in the Connaught Laboratories on the destruction of insulin in blood, the way in which heparin would behave in a reaction between trypsin and Insulin, attracted my interest, firstly, because the work on heparin was going on in the laboratory and secondly because Ferguson[11] has shown that different amounts of heparin can inhibit clotting of citrated dog plasma by crystalline trypsin under varying conditions of calcium and cephalin mobilization A study of the action of the Schmitz-inhibitor on the coagulation of blood plasma by trypsin, would be exceedingly interesting The effect of adding this inhibitor to a mixture of insulin and trypsin on the course of the destruction of the hormone has also been studied

Materials and Methods

Preparation of the trypsin-inhibitor from plasma (according to Schmitz) — 5 Litres of oxalated blood after keeping overnight in a refrigerator are mixed with 15 litres of 0 25 NH_2SO_4 to dissociate ferment inhibitor compound The dark coloured solution is kept overnight on ice 242 gm of $(NH_4)_2SO_4$ per litre (40% saturation) are then added The total albumins are precipitated The precipitate is filtered and thrown away To this yellow coloured filtrate, 205 gm of $(NH_4)_2SO_4$ per litre are added When this is kept for a few days in an ice-chest a fine flocculent precipitate separates It is filtered under suction on a hardened filter-paper The precipitate which is very small in quantity is dissolved in a few c c water (6 c c), the turbid solution is mixed with 4 c c saturated $(NH_4)_2SO_4$ solution and filtered The filtrate is salted out by the addition of 2 gm of $(NH_4)_2SO_4$ per 10 c c The white precipitate is filtered by suction on small hardened filter-paper The precipitate is dissolved in 5 c c water and the solution is mixed with equal volume of 5% trichlor-acetic acid This is kept for 30 minutes and the precipitate formed during this period is filtered and thrown away The clear filtrate is brought to pH 3 0 by the addition of a few drops of 5 N NaOH The inhibition body is then salted out by the addition of 5 6 gm ammonium sulphate per 10 c c The precipitate is filtered through a hardened filter-paper A fairly good quantity of the inhibitor (about 100 mg.) was obtained by undertaking the preparation in four different batches

Method of insulin assay employed —The mouse convulsion method was employed Since we were concerned only with comparative effects, a large number of animals were not considered necessary for the test On an average, 50 mice were used, 25 for test solution and 25 for standard.

Method of study of the prolongation of hypoglycemic effect —These tests were made by studying the effect of the test solution on the blood sugar of rabbits and comparing with the blood sugar reducing effect of a similar dose of standard insulin at the end of the same period The mean blood sugar figure for each hour is calculated both for rabbits injected with the standard and for rabbits injected with test solution Each figure is then converted to a percentage of the mean initial figure The point at which the blood sugar has returned to normal in the case of rabbits injected with standard insulin is taken At this point the blood sugar of rabbits receiving the test solution is noted The percentage of this value to the initial blood sugar gives an idea of the prolongation of the hypoglycemic effect when compared with standard insulin

The effect of the addition of the inhibitor isolated from plasma, on the coagulation of citrated plasma by trypsin, has been studied first In order to be able to study this, it is imperative to determine the optimum concentration of trypsin required to bring about coagulation in the shortest time The importance of this has been pointed out by Eagle and Harris[18] who have determined such an optimum concentration using Digestive Ferment Company's trypsin 1 110 and in the absence of calcium Ferguson and Erickson[13] have shown that the enzyme trypsin is much more potent in the presence of ionised calcium As it was intended to study the effect of the trypsin-inhibitor on the coagulation of citrated plasma by trypsin in the presence of calcium, it was considered of interest to find out the optimum concentration of the trypsin employed, (B D H) that is necessary to bring about coagulation of citrated plasma in the presence of calcium Accordingly the following experiments were undertaken —

TABLE I

Coagulation time of 1 c c plasma —varying quantities of trypsin solution (B D H trypsin 10% in salt solution)

Quantity citrated plasma	Quantity of CaCl₂N/10	Quantity of trypsin solution	Clotting time
c c	c c	c c	secs
1	0 25	0 75	32
1	0 25	0 60	19
1	0 25	0 50	35
1	0 25	0 40	80
1	0 25	0 30	95

The total volume was made up to 2 c c in each case

B1a

It is evident from the above table that 0 6 c c of a 10% solution of the trypsin used, coagulates 1 c c. citrated plasma in the shortest interval of 19 seconds in the presence of $CaCl_2$. Having thus determined the optimum amount of trypsin, we proceeded to study the effect of the Schmitz-inhibitor, isolated from plasma on the above process

TABLE II

B D H Trypsin—10% solution in 0 85% NaCl 5 mg of the trypsin-inhibitor dissolved in 10 c c of normal saline

Citrate plasma	$CaCl_2 N/10$	Trypsin	Inhibitor solution	Clotting time
c c	c c	c c	c c	secs
1	0 25	0 6		22
1	0 25	0 6		15
1	0 25	0 6	0 5	45
1	0 25	0 6	0 65	48
1	0 25			90

The volume was made up in each case to 2 5 c c

In the above experiments, the solutions were mixed in the following order Plasma—Trypsin—Inhibitor—Calcium

There was thus no interval allowed for the combination of trypsin and the inhibitor The inhibition brought about in the above experiments does not appear to be complete The maximum inhibition that has taken place (column 4) is still far from complete as can be seen from the blank experiment carried out with neither trypsin nor inhibitor As it is known that the inhibition of the trypsin brought about by this inhibitor is not instantaneous, and a certain amount of time is required to effectively block the enzyme, it was decided to incubate the enzyme solution and the inhibitor solution for a period of 1 hour at 30° C This incubated mixture was used in the following experiments

TABLE III

Plasma	$CaCl_2 N/10$	Trypsin + Inhibitor solution (a) (5 c c Trypsin + 5 c c Inhibitor sol containing 2 5 mg inhibitor)	Trypsin (10% Sol)	Clotting time
c c	c c	c c	c c	secs
1	0 25			95
1	0 25		0 6	22
1	0 25	1 2		70
		Trypsin + Inhibitor solution (b)	(5 c c Trypsin + 5 c c Inhibitor sol containing 5 mg)	
1	0 25	1 2		82
		Trypsin + Inhibitor solution (c)	(5 c c Trypsin + 5 c c Inhibitor sol containing 10 mg)	
1	0 25	1 2		85

When the inhibitor is incubated with trypsin before use, the blocking of the effect of trypsin on coagulation is more effective The amount of the inhibitor required to check the trypsin completely can be calculated from solution (b) 0 6 mg will prevent practically completely, 0 6 c c of 10% B D H trypsin from exerting its catalysing effect on the coagulation of citrated plasma. It is therefore clear that the plasma-inhibitor has got the property of inhibiting the coagulation action of trypsin Ferguson[11] has reported that heparin can inhibit clotting of citrated dog plasma by crystalline trypsin The plasma-inhibitor can therefore be considered analogous to heparin in this respect

The close analogy of thrombo-plastin and trypsin has been observed by Ferguson and fresh evidence has been adduced in support of this by Iyengar in his work on plasma-trypsin and prothrombin In view of this similarity of the behaviour of thrombo-plastin and trypsin in the process of coagulation, and in the light of the above results, the question whether the plasma-inhibitor can inhibit the thrombo-plastic action of the Russel viper venom, suggested itself to the author

The following experiments were carried out to test this possibility.—

TABLE IV

Oxalated plasma	Russel Viper Venom	CaCl₂N/40	Clotting time
c c 0 2	1 in 20,000 Russel Viper Venom 1 in 20,000 plus inhibitor (3 c c Venom sol plus 2 mg inhibitor) kept for one hour at 23° C	c c 0 2	secs 14
0 2 0 2	0 2cc nil	0 2 0 2	15 80

It is evident from the above table that the plasma-inhibitor cannot prevent the thrombo-plastic activity of Russel viper venom In this respect, the similarity between trypsin and thrombo-plastin breaks down

The destruction of insulin by blood has been investigated by a large number of workers (Schmdtz,[14] Karelitz,[15] Fraudenberg[16] and Black[17]). The destructive principle has been shown to be a proteolytic enzyme of a tryptic nature (Iyengar and Scott[18]) The presence of the anti-tryptic agents in blood assumes an added significance in the light of this property of blood. Advantage of the anti-tryptic effect of blood serum has been taken by Murlin and Hawley[18] to protect the insulin from destruction by trypsin and they claim that the hormone can be absorbed from alimentary tract of depancretized dogs Harned and Nash[19] have reported that when

insulin is mixed with anti-trypsin prepared from the round worm of swine (*Ascaris lumbricoides*), and given by stomach tube, it causes a marked decrease in sugar output These authors have also been able to demonstrate that the physiological activity of insulin can be protected from destruction by trypsin, if the enzyme has been previously incubated with this anti-trypsin

The possible prolongation of insulin action by inhibitors of proteolytic activity has not been studied by any of the above workers, but is merely mentioned by Horwitt in his work on heparin as an anti-tryptic agent. Since the inhibitor we are working with occurs in blood, it would be exceedingly interesting to study the (1) *in vitro* effect of a mixture of trypsin and inhibitor on the course of the destruction of insulin, and (2) the *in vivo* effect of injecting into rabbits a mixture of insulin and the inhibitor and observe the prolongation if any, of the hypoglycæmic effect as compared with standard insulin of an identical dosage The following experiments have therefore been conducted

TABLE V

Inhibition of the tryptic digestion of Insulin by plasma-inhibitor
The solutions were incubated for 2 hrs at 38° C
The pH of the solutions were maintained at
pH 8 by the addition of Phosphate buffer

	Increase in N P N mg	Destruction of Insulin Protein %	Destruction of Insulin Activity %
Trypsin + Insulin (5 mg. or 110 units)	0 32	40	90
Trypsin + Inhibitor (3 mg.) incubated for 1 hr and then Insulin (5 mg) added	0 08	10	40

The influence of the inhibitor on the course of the destruction of physiological activity of insulin and also on the digestion of the insulin protein has been studied in the above table. The digestion of the insulin protein is only 10% in the presence of the inhibitor whereas 40% digestion takes place if the trypsin is not previously incubated with the inhibitor While there is a definite inhibition of the tryptic destruction of the physiological activity of insulin, the inhibitor has not been able to prevent it completely It is generally recognised that a large part of the activity of insulin is destroyed during the early stages of digestion of insulin protein The fact that 40% of the activity is destroyed even in the presence of the inhibitor shows that the plasma-inhibitor is not capable of blocking the earliest stages of tryptic action Nevertheless the results obtained clearly show that the inhibitor checks the tryptic digestion of insulin protein

Having thus established that the plasma-inhibitor can partially block the tryptic destruction of insulin, the possibility of the practical applications of this finding was next investigated If the hypoglycæmic effect of insulin could be prolonged by the simultaneous injection of the inhibitor, it would be of great practical utility in the treatment of diabetic subjects The amount of the inhibitor circulating in the blood is not enough to block all the trypsin present in blood In addition the trypsin present in platelets (Iyengar and Scott) is released into the blood If therefore an additional amount of the inhibitor is administered with insulin, the hormone may not all be destroyed so quickly as it normally happens The method of study of the prolongation of the hypoglycæmic effect has already been described

TABLE VI

Effect of Plasma-inhibitor in the prolongation of the Hypoglycæmic effect of Insulin

Expt No		Blood sugar 6 hrs after injection expressed as percentage of the original blood sugar level
1	1 Unit Insulin injected	100
2	1 " " "	90
3	1 " " "	94
4	1 " " + 2 mgs Inhibitor	95
5	1 " " + 4 "	100
6	1 " " + 5 "	90

There is practically no prolongation of the hypoglycæmic effect since in all cases with varying amounts of the inhibitor, the blood sugar has been restored to the original level within a period of 6 hours after the injection of the mixture

Similar experiments were conducted using heparin in place of the inhibitor, in view of similarity of its behaviour towards trypsin.

TABLE VII

Inhibition of tryptic digestion of Insulin by Heparin

Solutions were incubated for 2 hrs. at 37°C	Increase in N P N	Percentage of destruction of Insulin Protein	Approximate percentage of the destruction of physiological activity
	mg		
Trypsin + Insulin (5 mg.)	0 30	36	90
Trypsin + Heparin (3 mg.) incubated for 1 hr at 37°C and then Insulin (5 mg) added	0 18	22	60

TABLE VIII

Study of the effect of Heparin in the prolongation of the hypoglycæmic effect of Insulin

Expt No		Blood sugar 6 hrs after Injection expressed as percentage of the original blood sugar level
1	1 Unit Insulin	100
2	1 ,, ,,	90
3	1 ,, ,, +2 mg. Heparin	94
4	1 ,, ,, +4 ,, ,,	90
5	1 ,, ,, +6 ,, ,,	95
6	1 ,, ,, +6 ,, ,,	92

Heparin is also not able to prolong the hypoglycæmic effect of insulin Although the trypsin-inhibitors are able to check the *in vitro* destruction of insulin by trypsin, they are practically useless in slowing down the process of physiological destruction of insulin in the body This is probably due to the fact that the trypsin present in blood or generated in the blood comes into contact with the insulin first and the destructive process starts before the enzyme can combine with the added inhibitor to form the inactive trypsin-inhibitor compound If the substrate and the inhibitor are allowed to come into contact with the enzyme simultaneously, the competition between the two for affinity with the enzyme comes into play In this process of competition, the substrate gets the upper hand If on the other hand the inhibitor alone is added to the tryspin and incubated for some time an inactive trypsin-inhibitor compound is formed

Crafford and Jorpes[30] observed that a larger doze of heparin is rendered inactive in the blood shortly after a surgical operation than is the case with the same patient before the operation This is regarded by them as a clear expression of the tendency to the formation of clots which cause thrombo-embolic complications post-operatively

The observation that trypsin and heparin are mutually antagonistic and that heparin inactivates trypsin both in its digesting and coagulating action, can be linked up with the finding of Crafford and Jorpes So far the only known antiheparin agents present in the blood are prothrombin and trypsin During the early stages of the post-operative period prothrombin is known to decrease if at all any change takes place in the prothrombin content of blood The changes in the trypsin content of plasma have not been studied The following table gives the tryspin content of plasma both immediately before and after the operation.

TABLE IX

Nature of operation	Plasma-trypsin before operation expressed as increase in N P N for 100 c c plasma	After operation
Obstructive Jaundice	mg. 22 5 18 4 24 8	mg 35 8 31 9 41 2

There is a significant increase in plasma-trypsin immediately after an operation. The increased inactivation of heparin under this condition may reasonably be ascribed to the trypsin content of plasma

Summary and Conclusions

A review of the anti-tryptic components of blood has been made There are present in blood three different substances capable of inactivating trypsin There are (1) A factor in serum associated with the albumins (2) An inhibitor isolated from plasma which according to Schmitz is a polypeptoid of low molecular weight (3) Heparin, the physiological anti-coagulant.

The *in vitro* effect of the plasma-inhibitor on the action of trypsin in catalysing the coagulation of blood has been studied in detail This substance has been found to inhibit this property of trypsin also

The course of tryptic digestion of insulin in the presence of this inhibitor and heparin has been studied both by following the increase in N P N, and also the physiological activity of insulin The digestion of the insulin protein is not completely checked although a definite inhibition is observed Since the digestion of insulin takes place even in presence of the inhibitor, or heparin, quite a considerable amount of physiological activity of insulin is destroyed by trypsin even in the presence of the inhibitor, or heparin This destruction is however very much less than the inactivation of insulin by trypsin under identical conditions but without inhibitor or heparin The plausible reasons for this observation have been enumerated

In view of the close analogy between trypsin and thrombo-plastin in their behaviour towards the process of coagulation, the action of the plasma-inhibitor on the thrombo-plastic activity of Russel Viper Venom was studied The thrombo-plastin of the venom remains quite active even after incubation with the inhibitor In this respect therefore the analogy between trypsin and thrombo-plastin breaks down.

Attempts have been made to ascertain whether prolongation of the hypoglycæmic effect of insulin can be obtained by injecting a mixture of insuln with the plasma-inhibitor or heparin A large number of experiments have been carried out to test this important practical application. The results obtained are not very encouraging and there is practically no prolongation of the effect either with the inhibitor or with heparin

The trypsin content of plasma have been estimated both before and after operation in a number of cases A significant increase in the enzyme content of the plasma has been observed immediately after operation It is suggested that this high trypsin content might be responsible for the increased inactivation of administered heparin, observed by Crafford and Jorpes

Acknowledgment

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PROTHROMBIN AND PLASMA TRYPSIN

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IT is now generally recognized that the coagulation of blood fibrinogen is brought about by thrombin produced from plasma prothrombin by the action of thrombo-plastin and calcium Prothrombin is present in the blood stream in a soluble and inactive form The exact nature of prothrombin has not been elucidated, but its preparations contain globulin protein which may be precipitated by dilution and acidification, or removed from plasma by colloidal adsorption Like other plasma proteins, prothrombin is formed in the liver but with the help of vitamin K

Mellanby[1] has prepared highly active prothrombin from beef blood and described its properties. It has been found to give well marked protein reactions Being a protein in nature, it would be naturally of great interest to study the action of proteolytic enzymes on prothrombin Mellanby however does not appear to have conducted any experiments to test the action of proteolytic enzymes, since he found that prothrombin is rapidly destroyed at 38° C in concentrations of acid and alkali in which pepsin and trypsin act

Pope[2] has demonstrated the action of pepsin and trypsin on plasma proteins at a pH, away from the optima of the action of these enzymes The enzymic degradation of the proteins may not take place but there is a possibility of the disaggregation which can be demonstrated by a change in the properties such as critical denaturation temperatures, etc In the case of prothrombin, since it is shown that its biological activity is very labile, any slight action on the prothrombin protein may be reflected in its biological activity The biological activity referred to is its capacity to form thrombin, in the presence of calcium and thrombo-kinase

Douglas and Co'ebrook[3] reported that blood coagulation was accelerated by the addition of trypsin Wall-Schmidt-Leitz[4, 5] and his co-workers also reported that trypsin accelerated blood coagulation They came to the conclusion that thrombin was a proteolytic enzyme either identical with, or related to, trypsin and that coagulation was due to the enzymic hydrolysis of fibrinogen to an insoluble modification Trypsin is considered to have

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accelerated coagulation in so far as it hastened this hydrolysis   Other proteolytic enzymes like papain were found to be active   Eagle and Harris[6] have made a detailed study of the action of trypsin on blood coagulation, in order to throw some light on the mechanism of physiological coagulation. Trypsin does not coagulate fibrinogen but apparently reacts with plasma prothrombin to form the physiological coagulant thrombin   Papain directly acts on fibrinogen to form an insoluble modification resembling fibrin.   It appears therefore that trypsin acts as a thrombo-plastic enzyme in assisting blood-clotting   The possibility of trypsin being a thrombin can be discounted in view of the complete inability of the former to clot fibrinogen meticulously free from prothrombin (Ferguson[7]) even when calcium and cephalin are also added   The possible rôle of trypsin in bringing about the activation of prothrombin solutions has been brilliantly discussed by Ferguson[8]   Prothrombin solutions used in his experiments were found to contain 8 to 30 mg. per cent of phospholipid   This amount of phospholipid if supplied in the form of added cephalin solution would be powerfully thrombo-plastic, while prothrombin solutions containing phospholipid in firm combination with proteins require the mediation of trypsin for the formation of thrombin   It is therefore concluded by Ferguson that trypsin splits off cephalin from its inert protein combinations and make it ' available ' for the activation of prothrombin   In the words of Ferguson, the action of trypsin consists in the " mobilization of cephalin and calcium at the colloidal surface of the protein (prothrombin) substrate where the close juxta-position of the three components permit of the formation of thrombin *via* an intermediary prothrombin-calcium-cephalin complex or compound "   According to this concept, the tryptic action is brought into line with the classical processes of thrombin formation   Schmitz[10] and Iyengar and Scott[11] have demonstrated the presence of trypsin in blood plasma.   One can therefore consider plasma-trypsin as part of the normal physiological clotting mechanisms.

A study of the action of plasma-trypsin on prothrombin at pH 7 2, the physiological pH of blood, will therefore throw light on the formation of thrombin on the one hand and also on the inactivation of prothrombin on the other   The present work was undertaken to elucidate the rôle, if any, of plasma-trypsin, in the physiological clotting mechanism   Incidentally, it may also be possible to discuss in the light of the results obtained the mechanism of the synthesis of prothrombin

The experiments reported in this paper can be broadly divided into two groups   In view of the thromboplastic activity of trypsin, the possibility of the trypsin in plasma acting as the physiological thrombo-plastin circulating

in blood can be visualised, and experiments have been designed to test this hypothesis. In order to test this hypothesis, the clotting times of oxalated plasma on mere recalcification in a large number of samples of blood, from different human subjects as well as from other species, have been determined Simultaneously the trypsin content of each sample of plasma was also estimated Secondly, the action of plasma-trypsin on the prothrombin present in plasma has been studied, to see whether any enzymic degradation of the prothrombin protein is a necessary adjunct to the physiological inactivation of prothrombin activity Since prothrombin is rapidly destroyed at a temperature of 36° in a solution of pH 8 4, the optimum for trypsin, specific action of plasma-trypsin on the prothrombin protein could be evaluated, only if the enzyme action was allowed to take place at pH 7 2, a reaction at which prothrombin solutions are definitely known to be stable The action of high-grade commercial trypsin on purified prothrombin solutions has also been studied

Material and Methods

Preparation of purified prothrombin —This was prepared according to Mellanby. 2 Litres of freshly bled ox blood is well shaken in a bottle containing 20 c c of 20% neutral potassium oxalate, and the plasma separated by high-speed centrifugalisation The plasma is diluted with 10 vols of distilled water and brought to pH 5 3 (the iso-electric point of prothrombin) by the addition of 1% acetic acid The supernatant fluid is then poured off and the dilute suspension of globulin is then centrifuged The precipitate is then resuspended in distilled water, equal to half the volume of the original plasma Dilute calcium bicarbonate solution is now added to an equal volume of the globulin suspension, mixed by gentle shaking and allowed to stand for about 10 minutes The suspension is rapidly filtered through a series of coarse filter-papers The prothrombin is precipitated from the rest of the solution by the addition of 1% acetic acid until the pH is approximately 5 3 The precipitate is then centrifuged, and the mass is quickly dried by treatment with acetone

Preparation of fibrinogen.—This was prepared according to Eagle and Harris Repeated precipitations with 1 5 vols of saturated NaCl yielded a satisfactory product This failed to coagulate on the addition of Ca and tissue extracts but was promptly coagulated on the addition of thrombin The final product was brought to a concentration of 0 9% with respect to NaCl by proper dilution

Trypsin.—Digestive Ferments Company's trypsin 'Trypsin 1 110' was used in the experiments in which the proteolytic action of purified prothrombin solutions was studied.

Estimation of prothrombin—After many trials with Quick[9] and Fullerton[10] techniques Iyengar *et al*[17] have evolved an improved and convenient method for the determination of prothrombin time This consists in adding to 0 2 c c plasma maintained at 38° C, 0 2 c c of a solution of 1 in 20,000 Russel viper venom in 0 025M CaCl₂ solution The interval between the addition of the calcium thrombo-plastin solution and the first appearance of the fibrin web is taken as the prothrombin time The shorter this interval, the greater the prothrombin content

Estimation of plasma trypsin—2 c c plasma was precipitated with 8 c c acetone, and centrifuged The precipitate was washed with acetone and allowed to dry for a few minutes The whole precipitate was then mixed up, with 10 c c of phosphate buffer of pH 8 4, the optimum for plasma-trypsin (Iyengar[11] and Scott) in a glass pestle and mortar to get a uniform suspension. 3 c c of this suspension was pipetted to a test-tube containing 3 c c of 10% trichlor-acetic acid The contents are well shaken and filtered The nitrogen in 3 c c filtrate is estimated by the micro-Kjheldal method Few drops of toluene are added to the remainder of the suspension and incubated at 37° C for 48 hours At the end of this period, 3 c c of this suspension is mixed with 3 c c of 10% trichlor-acetic acid, filtered and the nitrogen in 3 c c of the filtrate estimated The increase in non-protein nitrogen is taken as a measure of tryptic activity of plasma It is seen that by the above method, the free trypsin, in plasma, is precipitated by acetone along with plasma protein If the solids are suspended in the buffer of pH 8 4, and incubated, the autodigestion of the protein takes place giving rise to an increase in non-protein nitrogen Tryptic activity is expressed in terms of increase in N P N for 100 c c of blood plasma

Although the measurement of tryptic activity is carried out at pH 8 4, its action on prothrombin has been studied at pH 7 2 for the reasons enumerated in the early part of the paper While we were engaged in a routine determination of prothrombin levels in plasma of a large number of cases, the clotting time of plasma on mere recalcification without the addition of thrombo-kinase, was noted in every case It was found that this varied over a wide range while the prothrombin time itself with the addition of an optimum amount of thrombo-kinase, was remarkably constant with very slight variations Can this variation be due to the varying amounts of trypsin present in blood plasma ? This has been put to test and the results are given below.

Experimental

TABLE I

Relationship between ' Clotting time on mere recalcification of plasma ' and the free trypsin content of plasma

Human subjects	Case No	Prothrombin time	Clotting time on recalcification of plasma	Trypsin content as measured by increase in N P N calculated for 100 c c plasma
		secs	secs	mg.
Human subjects	1	10	45	23 5
,,	2	10	42	22 8
,,	3	9	35	24 2
,,	4	11	65	25 8
,,	5	10	60	19 2
,,	6	13	90	13 4
,,	7	9	65	18 9
,,	8	10	120	10 8
,,	9	8	45	23 5
Rabbit	10	7	35	28 5
,,	1	7	45	25 2
,,	2	8	65	21 4
Monkey	3	9	45	22 8
,,	1	2	45	21 4
Dog	1	9	60	18 3

It will be seen from the table that there is an inverse relationship between the clotting time on mere recalcification and the trypsin content of plasma. This can be noticed only when the clotting time is radically changed as for instance from 45 secs to 60 secs or to 90 secs or to 120 secs. In these instances there is a significant drop in the trypsin content of plasma. Minor changes in clotting time as for instance from 45 to 42 or 35, are not reflected in the trypsin content. In fact, even the tendency may sometimes be in the opposite direction. Such minor changes can only be ascribed to experimental error in trypsin determination, as we are dealing with minute quantities of trypsin from only 2 c c plasma. The figures in the table are however magnified since they are calculated for 100 c c plasma.

The action of plasma-trypsin on the prothrombin also present in plasma was next studied. The institution of blood banks in many of the larger hospitals has made the clinician largely dependent on stored blood for transfusion. It is now becoming increasingly apparent that such blood is not equivalent in all respects to freshly drawn blood. The normal blood contains a great excess of prothrombin beyond the amount necessary for clotting. The work of Quick[14, 15] has proved a rational basis for the belief that transfusion may diminish certain hæmorrhagic tendencies, associated

with prothrombin deficiency Prothrombin content of blood stored in the blood bank has been estimated by Rhoad and Panzer[14] at various intervals Their results have clearly shown that, although the blood is stored at 4° C in a sterile condition, the prothrombin content is gradually decreased In a week or more, the blood would be practically useless in the treatment of the acute prothrombin deficiency The cause of this spontaneous deterioration of prothrombin in blood is not known Dilute solutions of purified prothrombin appeared to maintain their biological activity quantitatively when stored under identical conditions as in a blood bank This has been indicated by Mellanby in his experiments on dialysis of purified prothrombin solutions, although the conditions of dialysis are not given The complete stability of such purified prothrombin solutions kept at a temperature of 4° under sterile conditions, was confirmed by us On account of the trypsin associated with prothrombin in plasma, the possibility of tryptic action on the prothrombin protein will have to be considered in this connection It may be pointed out that trypsin in such low concentrations as is present in plasma and at such a low temperature as 7° C and at a pH of 7 2, slightly removed from the optimum pH of trypsin, cannot be expected to have any action on the prothrombin protein Pope has shown that even under conditions approaching to the above, a disaggregation of the plasma proteins takes place Minor degrees of protein cleavage or perhaps mere intramolecular rearrangement may take place even under these conditions Mellanby has shown that protein is an acid meta-protein or is associated with an acid meta-protein, upon which the preservation of its properties depends Prothrombic activity is such a delicate property of the protein that any slight change brought about in the protein, may result in the deterioration of its potency That the trypsin in plasma may be responsible for the reduction of prothrombin in stored blood, can therefore not be rejected summarily

Prothrombin time of plasma was first determined immediately after the blood was taken The plasma was then kept in the frigidaire at a temperature of 7° C and the prothrombin time determined after different intervals Simultaneously with the prothrombin determination non-protein nitrogen in the plasma was estimated in order to see whether destruction of prothrombin is associated with an increase in N P N as a result of tryptic action In some cases, the plasma was incubated at 37° C and the prothrombin destruction and changes in N P N were followed at shorter intervals. In order to ascertain if the rate of prothrombin destruction has any relationship with the trypsin content of plasma, the trypsin was estimated in plasma by the method given above, after autodigestion for 48 hours. The results are given below

TABLE II (a)

Effect of incubation of plasma at 7° C and 30° C for different periods, on ` pro-
thrombin time ' and corelation between changes in ` prothrombin time `,
increase in N P N and the free trypsin content of plasma

Case No	Prothrombin time imme-diately	Prothrombin time after different intervals Plasma kept at 7° C			Increase in N P N			Trypsin in 100 c c plasma
		1	2	3	1	2	3	
	secs.	secs	days	secs.				mg
1	9	10	13	18	nil	nil	nil	23 2
2	8	11	17	25	nil	nil	nil	26 2
3	12	12	15	18	nil	nil	nil	18 5
4	14	14	19	22	nil	nil	nil	17 2
5	13	19	25	42	nil	nil	nil	38 4

TABLE II (b)

Case No	Prothrombin time imme-diately	Prothrombin time after different intervals Plasma incubated at 30° C			Increase in N P N			Trypsin in 100 c c plasma
		1	2	3	1	2	3	
	secs.	hrs 6	hrs 24	hrs 48	hrs 6	hrs 24	hrs 48	mgm
		secs	secs	secs				
1	10	13	25	40	nil	37	51	18 9
2	11	18	23	35	nil	42	62	22 5
3	8	10	19	did not clot for 4 minutes	nil	58	75	31 2

The results in Table II clearly show that plasma-trypsin slowly inactivates the associated prothrombin even when kept at 7° C There is no increase in non-protein nitrogen even after a period of 3 days, which indicates that the cleavage of the prothrombin protein has not taken place Since prothrombin activity has been affected, it should be assumed, that some change of the prothrombin protein has been brought about by the trypsin, which cannot be detected by the ordinary chemical methods employed in studying enzymic degradations of proteins   The biological activity of prothrombin serves in this case as an excellent method of detecting even the most superficial change involving perhaps intramolecular rearrangement of the protein   When the plasma is incubated at 30° C the prothrombin destruction is considerably accelerated This is as should be expected since the tryptic activity is enhanc-ed by the higher temperature   At the end of 24 hours, and 48 hours, there is

a definite increase in N P N also and the quantity of prothrombin destroyed is also greater   There is also a very rough relationship between the rate of destruction of prothrombin and the trypsin content of plasma.

In case (3) Table II (b) if purified prothrombin was added to the plasma after incubation for 48 hours, the prothrombin time was immediately restored This clearly shows that in these experiments, it is the prothrombin that has been affected and not any other clotting factor like fibrinogen

Finally the action of commercial trypsin on purified prothrombin protein has been studied.

200 mg   was dissolved in 250 c c   water and brought   to pH 7 2 by the gradual addition of a dilute solution of sodium carbonate and the volume was made up to 50 c c   To 25 c c of this solution was added 5 c c of 10% solution of trypsin and incubated at 37° C   The N P N was determined immediately and at definite intervals   The prothrombin time of this solution was also determined both immediately and after stated intervals by diluting 1 c c of the digest to 100 c c with 0 9% saline, and activating 0 2 c c of this with 0 2 c c of a solution of 1 in 20,000 Russel viper venom in 0 025 M CaCl₄ and then adding 0 2 c c of a solution of fibrinogen

TABLE III

Action of commercial trypsin on purified prothrombin

Time allowed	Prothrombin time	Increase in N P N in the digest
	secs.	mg
0	10	
15 minutes	16	
30 „	25	1 8
45 „	40	2 4
1 hr	62	3 5
2 hrs	Did not clot in 5 minutes	5 2

The results reported in Table III confirm the conclusion that the pro thrombin property of the protein is so labile that even slight intramolecular rearrangement can disturb the activity

Summary and Conclusion

The work of Northrop, and Kunitz,[12] Eagle and Harris, and Ferguson, has elucidated the rôle of trypsin in blood coagulation   Experimental evidence has been obtained to show that the trypsin in plasma may be the physiological thrombo-plastin   The thrombo-plastic action of trypsin depends on the amount present in plasma   Since the trypsin content of

plasma is very low, it is definitely below the optimum amount required to have the maximum effect Being present in sub-optimal amounts, the clotting time on mere recalcification is found to vary with the trypsin content The prothrombin time is constant irrespective of the plasma trypsin content, since during the determination of this factor, the optimum amount of thrombo-plastin is added in the shape of Russel viper venom Trypsin is reported to have (Eagle and Harris) no direct coagulative action on purified fibrinogen The coagulating action of trypsin was found to rest on the fact that it reacts with prothrombin to form thrombin This thromboplastic action of trypsin is observed only within a comparatively narrow optimum zone of trypsin concentration The trypsin in plasma is so small in quantity that it is highly probable that it is sub-optimal so far as its thrombo-plastic action is concerned Hence with varying concentrations of trypsin in plasma in the sub-optimal range, the clotting time of recalcified plasma is found to vary

The experiments on the incubation of plasma under sterile conditions at a temperature of 0° C and 30° C show that the gradual destruction of prothrombin activity takes place In the early stages, the destruction is not accompanied by any increase in N P N which is the earliest index of protein cleavage These results are suggestive of the possibility that mere intra-molecular changes of the prothrombin protein or mere disaggregation of the protein is enough to bring about a loss in prothrombin activity This is confirmed by experiments on the tryptic action of purified prothrombin solutions

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THE GRAIN SORGHUMS OF THE *DURRA* GROUP

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Introduction

Among the grain sorghums, the *Durras* form the most important group. Snowden[1] has recently made a systematic classification of the cultivated sorghums He has grouped the cultivated forms into six sub-series in a descending order of affinity to the wild types The sub-series *Durra* is the last of these six and the most removed from the wild ones. It consists of four species:—*Sorghum rigidum* Snowden, *Sorghum durra* Stapf, *Sorghum cernuum* Host, and *Sorghum subglabrescens* Schweinf et Aschers. Of these, the first named is a rare group and is reported to exist only in the Blue Nile district of the Anglo-Egyptian Sudan Moreover, it is less closely related to the other three species than they are among themselves. These three, *viz*, *S durra*, *S cernuum* and *S subglabrescens* form not only a compact, well-defined group, but are also culturally the most important among the grain sorghums This article gives a brief review of the *Durra* sub-series, with special reference to Indian and particularly to Madras varieties

Origin, History and Nomenclature

The origin, history and nomenclature of the species included in this sub-series have been fully reviewed by Snowden.

Chief Characteristics

The three important species of this sub-series are characterised by the following features. The plants are medium stout to robust with a coat of waxy bloom on the internode and leaf-sheath The panicle is usually compact, medium compact or medium loose (Fig. 1), and only very rarely loose. The rachis is stout and grooved, and the branches and branchlets are short, erect and hairy (Fig 2) The peduncle is usually erect except in

S cernuum where it is mostly goose-necked (Fig. 3) The sessile spikelets show a wide range in shape from ovate elliptic to rhomboid (Fig 4) The glumes are thick and spongy or thin and herbaceous. They are more or less equal in length. When herbaceous, there is often a transverse wrinkle about the middle of the glume (Figs 4 & 6) In the thickly coriaceous forms, there is no wrinkling and the tip is strongly nerved The lemmas are hyaline and ciliate, and most often have a long awn. The mature grains exceed the glumes in length and are as a rule readily separable The embryo mark is ovate to elliptic, flat or rarely concave The lateral lines are prominent. The endosperm is white in colour, mealy inside and hard towards the periphery Unlike most of the species of the earlier groups, e g., *Drummondii*, *Guineensia* and *Caffra*, the pedicelled spikelets in this are persistent (Fig 4) and have only short pedicels They are lanceolate to elliptic, and antheriferous or neuter The stalk is generally sweet

Sorghum durra Stapf

(a) *Characteristics.*

The plants of this species are generally stout-stemmed and broad-leaved The height varies from 125 cm in the Sudan and Sind varieties to 400 or even 450 cm in some of the Indian (Madras) varieties The duration ranges from 95–145 days The stalks are usually pithy in the ripe stage, but are mostly sweet The internode (fourth from the top) is 9–2·4 cm thick The number of leaves varies from 7–16 in the mature plant. The panicle is generally medium-loose to medium-compact, sometimes compact, and rarely loose, and 9 5–28 cm long and 5–17 cm broad The peduncle is erect as a rule, and is only rarely recurved. The heads are well emerged from the boot in the majority of types with a clearance of 10–15 cm The branches are rather rigid at the bases The racemes are somewhat crowded, mostly, three to four noded and fully hairy Sessile spikelets vary in shape from obovate-elliptic to rhomboid, and are 4 0–6 5 mm long and 2 5–5 0 mm. broad They may be red, black, buff or straw coloured, when dry The glumes are 4–6 0 mm long and 3–4 5 mm broad, and may be fully hairy or glabrate. The nodal bands may be fully hairy or glabrous (Fig. 5) The glumes are thickly coriaceous except at the tip, where it is thinly coriaceous and unevenly hairy The tip of the outer glume is broad, triangular and strongly nerved There are 12–16 primary and 6–8 secondary nerves on the lower glume The upper glume also has 7–9 primary and 5–7 secondary nerves, and is ciliate on the margins. The glumes are never wrinkled The outer lemma is ovate to broadly elliptic, 4 0–5 5 mm. long, 3–4 mm. broad and 2–5 nerved, and the inner is ovate and shortly two-lobed, with an awn up to 12 mm in length. The anthers are 3–4 mm long and

1 mm wide Grains vary in shape from obovate to broadly ovate or sub-rotund, and are 4 0–6 5 mm long and 3 0–6 mm broad, and have a broad rounded and much exposed top The majority of the types are yellow or white grained, red grained come next, while brown-grained types are somewhat rare. The pedicelled spikelets are lanceolate to linear oblong, 4–8 mm. long and 75–2 mm broad

This species is characterised by the presence of obovate-elliptic to rhomboid-sessile spikelet, thickly coriaceous glume with broad and strongly nerved herbaceous tip, awned or rarely mucronate inner lemma and biconvex grain with broad and round top and wedge-shaped base It differs from *S cernuum* in having more thickly coriaceous glume which is not wrinkled and which becomes more or less glabrous on the back at length. Moreover the grain in *S cernuum* It broadly elliptic to orbicular in shape and much more flattened than in *S durra* *S durra* can be distinguished from *S subglabrescens* in having thickly coriaceous and rather spongy glume which is comparatively more hairy and not transversely wrinkled as in the latter

(b) *Distribution —*

This species is now the most important grain sorghum in Egypt, the Anglo-Egyptian Sudan, Eritrea, Arabia and India In recent times it has also been introduced into the United States of America and in addition is also grown in parts of Middle and Western Asia, namely Iraq, Mesopotamia and Palestine In India *Durra* varieties are cultivated in almost all the provinces where sorghum is grown, particularly in Madras, Sind and Baluchistan It forms the chief rain-fed variety in the Coimbatore, Guntur, Cuddapah, Kurnool, Kistna, Godavari and Vizagapatam districts and occupies about 46 per cent of the total area (4 6 million acres) under sorghum in the Madras Presidency, *S subglabrescens* having 18 per cent and *S cernuum* 17 per cent Thus the species of the *Durra* sub-series occupy more than four-fifth of the total sorghum area in the Madras Presidency

(c) *Varieties —*

Out of the 16 varieties into which Snowden classifies *Sorghum durra* only nine, viz, *mediocre, coimbatoricum javanicum, fecundum, eois, elongatum, fuscum, rivulare* and *maximum* are found in India The remaining seven varieties, viz, *aegyptiacum, Floril, rutilum, niloticum, melanoleucum, erythrocarpum* and *luteolum* are confined to Anglo-Egyptian Sudan, Egypt and Eritrea Among the varieties cultivated in India, vars. *mediocre, coimbatoricum* and *javanicum* alone are grown in the Madras Presidency. A short description of these is given below

(i) Var *mediocre* (Burkill) Snowden.—This variety is confined to the districts of Northern Circars namely, Vizagapatam, Godavari and Kistna, the Ceded districts of Cuddapah, Kurnool, Bellary and Anantapur and the adjoining districts, viz., Guntur, Nellore and Chittoor It is known in the different districts under different local names such as *Bommayi jonna, Budda jonna, Desa jonna, Gidda jonna, Harasana Jola, Mallemari jonna, Napa jonna, Pacha jonna, Pairu jonna, Pedda jonna* and *Zinkapuri jonna* The *Pacha jonna* of the Circars and the Ceded districts is typical of this variety

Chief Characteristics—Types mostly rain-fed, plants medium tall, height 125-285 cm, duration 100-130 days, stalk 0 9-1·5 cm thick, pithy, leaves 10-14 in number, 50-70 cm long and 6 9 cm broad, sheath and glumes mostly reddish purple, axil purple, midrib white, panicle medium loose to medium compact, 10-25 cm long and 5-12 cm broad, awn long, glumes glabrous and slightly or fully bleached, grains pearly yellow, invariably with a characteristic brown wash, 4-5 5 mm long and 3-4 5 mm broad; pedicelled spikelets 4-6 mm long, and 75-1 5 mm broad and turns brownish yellow on drying. This is the chief sorghum grown in the Northern Circars But being a paddy tract, rice forms the chief cereal for food there, and sorghum is mostly exported In the Ceded districts and also in Guntur, Nellore and Chittoor this variety forms one of the chief food grains The yield ranges from 300-800 lb of grain and 900-1,500 lb of straw per acre

(ii) Var *coimbatoricum* (Burkill) Snowden.—As the name indicates, this variety is confined to the district of Coimbatore Recently it has spread to the neighbouring districts mainly through the endeavour of the Agricultural Department This variety is commonly known by the name *Periamanjal cholam* and is rarely called *Sadaimanjal* The name *Periamanjal* refers to the long duration, the great height and the yellow grain of this variety

Chief Characteristics—Plants are very tall (tallest among the Indian sorghums), stout and long in duration. Height 300-450 cm ; duration 130-40 days, stalk 1-2 cm thick, leaves 12-16 in number, 60-70 cm long and 8-10 5 cm. broad, leaf-sheath and glume reddish or blackish purple, node glabrous, axil purple, midrib white, panicle large, medium loose, 14-26 cm long and 7-17 cm broad, awn long, grain pearly yellow with a characteristic brown wash and somewhat duller in colour than in var *mediocre*, 4 5-5 5 mm long and 3 5-4 5 mm, broad and tightly held by the glumes. This variety is highly season-bound, the flowering period being

confined to the months of October and November   The yields vary from 600–1,200 lb of grain and 2,000–6,000 lb of straw per acre. As a food grain this is considered superior to the other varieties in the Coimbatore district.

(iii) Var *javanicum* (Hack) Snowden —This variety has a wider distribution than the two described above being found in all the sorghum growing provinces of India as well as in Afghanistan, Anglo-Egyptian Sudan, Egypt and in Morocco   In the Madras Presidency it is grown chiefly in the districts of Bellary, Anantapur, Chittoor and North Arcot. It is known under different local names such as *Hira jola, Konal cholam, Nagari cholam, Nettai jola, Nir jola, Vellai cholam* and *Vibhuthivantha jonna*   The *Vellai cholam* of Chittoor and North Arcot is typical of this variety   This consists of types which are grown both under irrigated and rain-fed conditions

Chief Characteristics —Height 140–75 cm in the irrigated and 165–225 cm in the rain-fed ones, duration 110–15 days in the irrigated, and 120–35 in the rain-fed types, stalk 0 9–1 6 cm thick and pithy, leaves 7–12 in number, 60–70 cm long and 6 5–9 cm broad, panicles compact, ovate to conical, 10–20 cm long and 6 5–10 cm broad, glumes mostly reddish purple, always fully hairy, 4 5–6 mm long and 2 5–4 mm broad, awn long, grain mostly white, 4–5 5 mm long and 3–5 mm broad. The yield varies from 400–1,000 lb of grain and 2,000–3,500 lb of straw per acre

Of the three varieties of *S durra* described above vars *coimbatoricum* and *mediocre* have yellow grains, and var *javanicum* is mostly white grained Var *coimbatoricum* differs from vars *mediocre* and *javanicum* in having much taller and more robust plants with larger panicles and bolder grains which are rather tightly held by the glumes   Moreover in this variety both reddish purple and blackish purple coloured leaf-sheaths are met with, while in mediocre and *javanicum* blackish purple is very rare   In var *javanicum* the panicles are more compact than in the other two

Sorghum cernuum Host

(a) Characteristics

Plants shorter than those of *S durra*, height 110–300 cm , duration 95–140 days; stalk 0 9–2 2 cm thick, mostly juicy and sweet, leaves 7–15 in number, 45–75 cm long and 5–10 5 cm broad; node green and fully hairy; axil of leaf-sheath purple, leaf-sheath and glume mostly reddish purple, blackish purple being rare, awn long, panicle compact to medium compact, 8–27 cm long and 5 5–19 cm broad, peduncle mostly recurved (Fig 3), rachis fully hairy and branches short and sub-erect, sessile spikelets broadly ovate to obovate-oblong and fully hairy , callus beard copious; glumes fully hairy to villous, equal, thick and spongy below the middle and

thin and papery above with a transverse wrinkle, creamy white to straw or buff in colour, 4-6 mm. long and 3-4·5 mm broad and tips breaking off at maturity, grain rotundate or orbiculate, flattish, 4-6 mm. long and 3·5-5 mm broad, protruding beyond the glumes, mostly white, occasionally red and rarely yellow in colour; pedicelled spikelets generally large, fully hairy and mostly antheriferous, turning red in red grained and straw coloured in white grained types.

The distinguishing characteristics of this species are the silky hairs on the nodes and the glumes, completely bleached glumes which are somewhat thick and spongy at the base, thin and herbaceous at the tip, transversely wrinkled or depressed at the middle, and breaking off at the tips, and the orbicular and flattened grains. This differs from *S. durra* in having more hairy and transversely wrinkled glumes and more flattened grains, and from *S. sub-glabrescens* in having fully hairy and completely bleached spikelets which are invariably long awned.

(b) Distribution

This species is less wide in its distribution than *S. durra* It is found in India, parts of Afghanistan, Persia, Arabia, Asia Minor, Egypt and British Somaliland. At one time it was extensively cultivated in Egypt, although it is now largely replaced by *S. durra* This species figures to a slight extent in the United States of America having been first introduced in 1874 under the name of white durra. In India it is limited chiefly to the uplands of the Deccan. Varieties of this species are grown in Rajputana, Sind, Bombay, Hyderabad, Central Provinces, Central Indian States, Mysore and Madras. A few types are found in Bihar and Orissa also. The plants of these varieties seem to do well only in areas with highly retentive clayey soils, low rainfall during the growing period, and an absolutely rainless, cool weather during the ripening stages. The ability of the plants of this species to resist drought is shown by the fact that it is distributed over the driest areas of this Presidency as well as of India in general. In the Madras Presidency *S. cernuum* occupies a predominant place only in the Ceded districts. It is of minor importance in the adjacent districts of Guntur and Chittoor. Though isolated areas occur here and there, where *S. cernuum* is grown under irrigation, it is mostly a rain-fed species.

(c) Varieties

Snowden has divided this species into seven varieties, viz, *truchmenorum, yemense, agricolarum, globosum, orbiculatum, subcylindricum* and *cernuum*, all of which occur in India, and two are confined to India alone in

their distribution. Of the above seven varieties only three namely, *agricolarum, globosum* and *orbiculatum* are cultivated in the Madras Presidency to a large extent, and a short description of these is given below

(i) Var *agricolarum* (Burkill) Snowden —Grown chiefly in the district of Bellary and portions of Kurnool this is known locally as *Yerra jonna* and *Yerrapusi jonna* which refer to the red colour of the grain. It is usually cultivated as a dry crop. Owing to the juicy stalk and the leafy nature of the plants this is considered as a good fodder variety and yields 10,000–15,000 lb of green fodder per acre. In extraction tests this gave 43·3 per cent. of juice with a Brix value of 12·6. This juice contained 6·6 per cent. of sucrose and 3·0 per cent of glucose

Chief Characteristics —Height 250–300 cm., duration 120–40 days, stalk 0·9–1·4 cm. thick, leaves 12–15 in number, 50–70 cm long and 6–8 cm broad with the margins turning red on drying, peduncle recurved in most cases, panicle compact, 9–15 cm long and 6–8 cm broad, grain red, very bold, 5·5 mm long and 4·5 mm broad with a dimple in rare cases, pedicelled spikelets 4–5 mm long and 1–2 mm. broad

(ii) Var *globosum* (Hack.) Snowden —The different forms of this variety are found chiefly in the districts of Cuddapah, Kurnool, Bellary, Anantapur and Chittoor. They are known by the names *Chitta jonna* and *Nallapusi jonna* in Kurnool, *Tella jonna* in Bellary, Cuddapah and Chittoor, and *Tella Thota jonna* in the district of Anantapur. They are mostly rain-fed types.

Chief Characteristics —Height 180–240 cm., duration 100–130 days, stalk 1–1·4 cm thick, leaves 10–14 in number, 50–75 cm long and 6·5–9·5 cm broad; panicle medium to very compact, 10–20 cm long and 7–10 cm broad, grain very bold, mostly pearly white, 4–5·5 mm long, and 3·5–5 mm broad, a few double grained due to the lower lemma being fertile, endosperm mealy white, pedicelled spikelets 4–6 mm long, and 1–2 mm. broad. This is the most important of the varieties of *S cernuum* cultivated in the Ceded districts. The yield is 300–600 lb of grain and 1,000–2,000 lb of straw per acre

(iii) Var *orbiculatum* Snowden —This variety has a much wider distribution than either of the two described above, but in Madras it is grown to a lesser extent and is mostly found in the districts of Bellary, Chittoor and Guntur. The types in this variety are known as *Belkalu jola* (white pearly grained) in Bellary, *Mudda* (ball-like) *jonna* in Chittoor and *Venna Mudda* (butter ball) *jonna* in Guntur. This is similar to var *globosum* in height, duration, and other plant characters, the chief point of difference being the more flattened nature of the grain in this variety.

The three varieties of *S cernuum* namely *agricolarum*, *globosum* and *orbiculatum*, generally met with in the Madras Presidency, have been described above. In these, var *agricolarum* consists of red grained types only and in this the spikelets and the margins of the leaves are reddish in colour, when dry In the varieties *globosum* and *orbiculatum* the majority of the types are white grained Variety *orbiculatum* can be distinguished from var *globosum* by the grain of the former being orbicular in shape and much more flattened than that of the latter

Sorghum subglabrascens Schweinf et Aschers.

(a) Characteristics

Height 70–335 cm , duration 85–135 days, stalks generally pithy, thinner than those of *S durra* and *S cernuum*, range in thickness being 0 8–1 5 cm , leaves 7–14 in number, 40–70 cm long and 4 5–8 cm broad, panicle compact to very compact and sometimes loose, 8–25 cm long and 5–12 cm. broad; peduncle generally erect and rarely goose-necked, branches and branchlets less hairy than in the other two species, sessile spikelets oblong to hexagonal in shape, 4–6 5 mm long, and 2 5–4 mm broad, often hairy when in flower, and ultimately glabrate, callus beard scanty; glumes 4–6·5 mm long and 2 75–4 mm broad, thick and papery except near the base, wrinkled or depressed about the middle and with 12–14 primary and 5–8 secondary nerves, awn long in the majority of types, grain white, yellow or red and rarely brown in colour, 3–5 5 mm long and 3–4 5 mm broad; with a rounded tip and an abruptly compressed, wedge-shaped base, yellow type invariably long awned, pedicelled spikelet small, and reddish in red and yellowish in yellow grained types

The distinguishing characteristics of this species are the semi-membraneous, glabrate glumes which are usually transversely wrinkled about the middle (Fig 4) and broad topped grains with abruptly tapering wedge-shaped bases The plants are usually shorter and less robust than in *S durra* and somewhat less robust than in *S cernuum S subglabrescens* can be distinguished from *S cernuum* by the glumes of the former being glabrous or less hairy and the grains biconvex and broad topped with abruptly tapering wedge-shaped bases It differs from *S durra* in having obovate-oblong to hexagonal spikelets, less hairy to almost glabrous, thinner and transversely wrinkled glumes, and abruptly tapering grain bases

(b) Distribution

This species is distributed in the Anglo-Egyptian Sudan, Eritrea, Abyssinia, Arabia and India It has also been introduced recently into South Africa, Nyasaland and the United States of America

(c) Varieties·

Of the 17 varieties into which this species is classified by Snowden, 10 are limited in their distribution to Africa, and of the remaining seven some are found both in India and Africa, and the rest in India only Of these seven varieties, *pabulare, rubidum, compactum, irungiforme* and *oviforme* are concentrated in the Madras Presidency, while *paniculatella* and *rugulosum* are reported from Bombay and the Central Provinces In Madras *S sub-glabrescens* is more abundant in the south than in the north where *S durra* and *S cernuum* predominate A short description of the varieties culti-vated in the Madras Presidency is given below

(i) Var *pabulare* Snowden —The word *pabulare* indicates fodder, and this variety is raised more as a fodder than a grain crop. Under the names of *Nilwa jowar* and *Utavil jowar* it is grown extensively in the Bombay Presi-dency and the Central Provinces In Madras it is not so prominent, and is represented only in two districts—Ramnad and Tinnevelly, where these are known as *Arisi cholam* or *Uppu cholam* These *Arisi cholams* have a great affinity to the *Irungu cholam (S dochna)* in having reed-like stalks, narrow leaves, large number of tillers, loose panicles and small grains which are almost completely enclosed by the glumes In these two districts the *Irungu cholam* being the most common variety grown for fodder, and the white grained forms of *S subglabrescens* for grain, it is quite possible that the variety *pabulare,* having the characteristics of both *S subglabrescens* and *S dochna* might have originated as a product of hybridisation between the white-grained forms of these two species. Similarly the *Nilwa jowars* of Bombay, Bihar and the Central Provinces show some of the characteristics of both *S cernuum* and *S subglabrescens* and seem to have been evolved through hybridisation between certain white-grained types of these two species

Chief Characteristics —Height 180–225 cm ; duration 95–105 days, stalk 0 8–1 cm thick, leaves rather stiff, 8–13 in number, 50–65 cm long and 4·5–5 5 cm broad, leaf-sheath and glumes either reddish or blackish purple, node, junction and glume completely hairy, panicle loose conical, 18–25 cm long and 8–12 cm broad, awn 8–10 mm long, grain chalky white and small; glumes extending nearly to the tip of the grain, slightly bleached and transversely wrinkled, pedicelled spikelets small and sterile This variety is of minor importance in the Madras Presidency

(ii) Var *rubidum* (Burkill) Snowden —This is one of the four important varieties of *S. subglabrescens* grown in the Madras Presidency Its distri-bution extends from Nellore in the North to as far as Madura in the South, being cultivated in Nellore, Guntur, North Arcot, Ceded districts, Salem,

Coimbatore, Trichinopoly and Madura Among these districts however, its greatest prominence is in Salem and Trichinopoly where large areas are grown under the name of *Sen cholam* The name *rubidum* has arisen from the word *rubidus* meaning reddish, and the grain in this is generally red or light red This consists of both irrigated and rain-fed types the latter being 50–100 cm greater in height and 20–30 days longer in duration than the former The most common names by which this variety is known are *Kunkuma jola, Makkattai cholam, Palapu jonna, Sakkaraguliga jonna, Sen cholam* and *Yerra jonna* The *Sen cholam* is the most typical of this variety

Chief Characteristics—Height 125–245 cm , duration 100–135 days , stalk 1–1 2 cm thick , leaves 8–12 in number, 50–60 cm long and 5–7·5 cm broad; leaf-sheath and glumes reddish purple, node and junction glabrous, panicle medium compact in rain-fed and compact to very compact in irrigated types, 10–14 cm long and 5–7 cm broad, peduncle usually erect and rarely recurved, glumes slightly bleached and wrinkled, awn long in most of the rain-fed types, and absent in the irrigated ones; and grain bold, red, light red or pink in colour The yield is 1,500–3,000 lb of grain and 3,000–5,000 lb of straw per acre under irrigated condition while the rain-fed crop gives 600–800 lb of grain and 2,000–3,000 lb of straw

(iii) Var *compactum* (Burkill) Snowden—This variety is found only in India where it is confined to the Central Provinces and the Madras Presidency In the latter province it is grown chiefly in the districts of Coimbatore, Trichinopoly and South Arcot, and to a small extent in Bellary and Anantapur It is known as *Azhukku cholam, Chinna* or *Chitral manjal cholam, Dosakaya jonna, Kullamanjal* or *Kullanari cholam, Manja makkattai* and *Sena jonnalu* The *Chinna manjal* and *Manja makkattai* are typical of this variety As in *rubidum, trungiforme* and *oviforme* the types in this variety also fall into two groups of duration, the shorter (95–110 days) grown under irrigation from March to June, and the longer (120–135 days) grown rain-fed from July to December The irrigated ones are 160–220 cm and the rain-fed ones 200–310 cm tall Other characteristics are.—stalk 1–1 4 cm thick , leaves 10–13 in number, 50–70 cm long and 7–8 cm broad; leaf-sheath and glumes reddish purple , nodal band, junction and glumes glabrous; panicle medium compact to compact, ovate, awn long , glumes obovate, blunt tipped, and wrinkled with tips breaking off at maturity, and grains pearly yellow with or without a brown wash In the *Chinna manjal cholam* the tissue at the nodal band develops a characteristic purple colour which presents a checkered or cracked appearance at the ripe stage, and this purple is linked with the sienna coloured dry anther and yellow grain without the brown wash The yields vary from 1,500–2,500 lb of grain and

3,000–5,000 lb of straw per acre in the irrigated, and 500–800 lb of grain and 2,000–4,000 lb of straw in the rain-fed crops

(iv & v) Var *irungiforme* (Burkill) Snowden and Var *oviforme* Snowden — These two varieties are similar in all morphological characters and cultural features. The only difference is that the var *oviforme* has a much denser and shorter panicle than the var *irungiforme* in which the panicle shape varies from compact to medium compact These two varieties are therefore discussed together. As already recorded, both the varieties are purely Indian in their distribution and the majority of types are found in Madras and a few reported from Bombay, Sind and the Central Provinces Both the varieties are known by a number of local names, the most common of these being *Chinna vellai, Ennai vellai, Kattai vellai, Kokki vellai, Kullanari cholam, Tella jonna, Uppam* or *Uppu cholam* and *Vellai cholam* The last named is perhaps the most typical of these. Variety *irungiforme* is more widely distributed in Trichinopoly district while var *oviforme* predominates in Madura. In some cases both the varieties are known by the same local name as *Kullanari cholam, Uppam cholam* and *Vellai cholam*

Chief Characteristics —Height 150–230 cm in the irrigated and 220–350 cm in the rain-fed ones, duration 90–110 days in the irrigated and 125–35 days in the rain-fed ones, stalk 1–1 2 cm thick, leaf-sheath and glume reddish purple or blackish purple, brown sheath being rare , leaves 10–14 in number, 50–60 cm long and 5–7 5 cm broad, node and junction glabrous in the majority of types, panicle compact to medium compact, 15–20 cm long and 8–10 cm broad in *irungiforme*, and more compact and 8–14 cm long and 5–8 cm broad in *oviforme*, glumes ovate to obovate, broad tipped, transversely wrinkled in the majority of types and 3 5–5 mm. long and 3–4 mm broad, and grains white, mostly pearly Both the varieties consist chiefly of irrigated types, the rain-fed ones being few and less important. The irrigated crop is raised from January to June and gives 1,500–3,000 lb of grain and 4,000–6,000 lb of straw per acre. The rain-fed is grown from July to February and produces an acre yield of 500–800 lb of grain and 2,000–4,000 lb of straw

The five varieties of *S subglabrescens,* namely, *pabulare, rubidum, compactum, irungiforme* and *oviforme*—cultivated in the Madras Presidency have been described above. Var *pabulare* differs from the others in having reed-like stalks, profuse tillering, loose conical-shaped, long panicles and small grains which are almost completely enclosed by the glumes. Var. *rubidum* is distinguished from the rest by its red or light red grains. Among the remaining varieties *compactum* has pearly yellow grain invariably asso-

ciated with a long awn, and *trungiforme* and *oviforme* are white grained Var. *irungiforme* differs from var *oviforme* in having more elongated and less dense panicles

Border Types

The three important species in the sub-series Durra have now been discussed There occur however certain types that appear to be intermediate between these species They cannot be included under any one of these three—*S durra, S cernuum* or *S subglabrescens*, and can only be classed as intermediate or border types Thus there are border types between *S durra* and *S cernuum*, between *S cernuum* and *S subglabrescens*, and also between *S durra* and *S subglabrescens* These border types are mostly found in regions where all the three species are simultaneously grown as in the Ceded districts In the other districts where either one species alone is grown or different species are raised in different seasons, these border types are rare, probably due to lack of chances for intercrossing The *Tella kugu jonna* of Anantapur and *Tella jonna* of Cuddapah are considered as border types between *S durra* and *S cernuum*, *Manadanti* and *Palumadi jonna* of Bellary and *Cherukku jonna* of Kurnool as border types between *S cernuum* and *S subglabrescens* and *Kakunari jonna* of Anantapur seems to be a border type between *S durra* and *S subglabrescens*

A Brief Review of Plant Characters met with in the Durra Group

Height and Duration—Most of the types in the Durra sub-series fall within the medium duration group, and have the unimodal disposition in their internodal lengths [8] The relationship between height and duration was studied in a mutant in a *Pacha jonna* type (*S durra* var *mediocre*) and the short early was a simple dominant to the tall-late [9]

Root colour—In this sub-series there is a preponderance of the reddish purple type, the blackish purple being few and the brown rare

Root system—Most of the varieties of the Durra sub-series have very well developed root systems This is more evident in the rain-fed than in the irrigated ones Some, particularly when desheathed, have a tendency to develop aerial roots [6]

Leaf-sheath—In this group the leaf-sheath covers more than the lower half of the internode, and the direction of the æstivation is normal, being alternately clockwise and anti-clockwise [8] Aberrations from this normal condition have been met with in a *Pacha jonna* type called *Edakula jonna* in which two to seven successive leaf-sheaths were clockwise or anti-clockwise in æstivation.

Leaf-blade —In the majority of the varieties in this group the leaf-blade is broad with smooth junctions and wavy margins[4] In *S durra* and *S. cernuum* most of the types have hairy leaf tips

Nodal band —This is generally green and mostly hairy in this group, the glabrous types being in a majority in *S subglabrescens* alone Purple coloured band is rare

Axil of leaf-sheath —Most of the types in this sub-series have purple coloured axil Green axils are very few and deep purple ones rare

Leaf-junction —In the majority of the varieties of this group the junction is generally green, hairy and smooth It is worth noting that hairy nodes and junctions are more numerous in the rain-fed than in the irrigated types

Midrib colour —In the *Durra* sub-series generally the midrib is white in colour and the stalk pithy but sweet In *S cernuum* the majority have juicy stalks Apart from juiciness or pithiness, the midrib in some types in all these species is yellow[2] and in a rare mutant in *S durra*, it is brownish purple[4] The colour in these cases is confined to the mechanical tissue The yellow is dominant and the brownish purple recessive to the colourless condition

Peduncle —In this sub-series the peduncle may be erect or recurved, the latter being in a majority in *S cernuum* In *S subglabrescens* a half recurved type is commonly met with in var *rubidum, compactum, trungiforme* and *oviforme* Warty protuberances are also met with in certain types and these are presumed to help in the liberation of the head from the boot

Emergence of panicle —The heads are fairly well emerged in *S durra* and *S subglabrescens*, but in *S cernuum* the emergence is poor

Panicle —In the *Durra* sub-series the panicle shape falls mostly in the ovoid group[9] with variations in the degree of compactness Within the ovoid group the medium compact behaved as a simple dominant to the compact type

Awn —The presence of awn is the most common feature in this group Of the three species, *S durra* and *S cernuum*-mostly rain-fed-consist almost entirely of long awned types, while in *S subglabrescens* both nil awned and long awned types occur, with the latter in slight excess especially in the rain-fed varieties As already recorded, the yellow grain is almost invariably associated with a long awn The awn is greenish in the flowering stages but the column takes on the tint of the glume on drying

Glume—In all the three species in this sub-series the glumes are thickly coriaceous at the base and thinly coriaceous to herbaceous at the tip. In *S cernuum* and *S subglabrescens* the herbaceous tip breaks and falls away at maturity Both the glumes (as in *S cernuum* and the majority of *S subglabrescens*), or one only (as in the rest of *S subglabrescens*), or neither (as in *S durra*) may be transversely wrinkled (Fig 6) Glume wrinkling commences in about 15 days after flowering, when the grains are in the dough stage, and completes in about 10 days, by which time the grain becomes fairly hard Wrinkling tends to desiccate the upper half of the glume, with the result that any colour present there gets bleached out, and hence in most of the types of this group the glume is partially or completely bleached. Wrinkling has been recorded as dominant to its absence,[10] but it is not stated whether one glume alone or both the glumes were wrinkled. In crosses between types with both the glumes wrinkled and no wrinkling in any glume the F_1 showed wrinkling on both the glumes, and the F_2 gave a 9 7 ratio of wrinkled to not-wrinkled A heterozygous mutant with both the glumes wrinkled occurred in an irrigated *Sen cholam* (upper glume alone wrinkled) and it gave a monogenic segregation between both glumes and upper alone wrinkled The relationship between the upper glume alone wrinkled and the complete absence of wrinkling, and other aspects are under study The glumes in all these varieties are short nerved In crosses with the long nerved types of *S nervosum* the short nerved condition behaved as a simple dominant [a]

Grain—White, yellow and red are the most common grain colours met with in this group, brown is rare *S durra* and *S subglabrescens* contain all the colour forms In *S cernuum*, on the other hand, white and red alone are usually met, yellow and brown are very rare The grain shape in this sub-series varies from ovate to rotund or orbiculate with a wide range in the size of the grain The largest grain so far met with in sorghum is found in var *niloticum* and *rivulare* of *S durra* and the most flat grain in the *Chapti Juar (S cernuum)* of the Central Provinces A few types in this group have dimpled grains This character was first noted in a type of *Sakkara-gulga jonna* of Bellary classified under *S. cernuum* Later on dimpled types were found to occur in varieties of *S subglabrescens* as well of this tract

Pedicelled spikelets—In this sub-series the pedicelled spikelets are elliptic to lanceolate or linear-oblong in shape, sparsely to moderately hairy, large, conspicuous and antheriferous, or small and neuter, and always persistent The pedicel is short In red and yellow grained varieties the margins of the pedicelled spikelets turn red and brownish yellow.

(a) *S. durra*   (b) *S. cernuum*   (c) *S. subglabrescens*
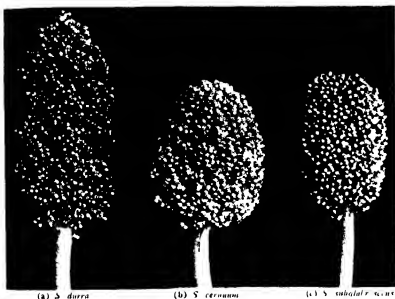
Fig. 1 Sorghum Panicles

Fig. 3 A Panicle showing recurved (goose-neck)

(a) Hairy   (b) Glabrous

Fig. 5 Nodal bands

(a) S aurea   (b) S cernuum   (c) S subglabrescens
Fig 2   Panicle Branches

(a) S aurea   (b) S cernuum   (c) S subglabrescens
Fig 4   Spikelets and Grains (Front and Side views)

(a) Both glumes   (b) Upper glume   (c) Both glumes
    wrinkled   alone wrinkled   not wrinkled
Fig 6   Glume Wrinkling

Chlorophyll colour grades—Most of the varieties of the *Durra* group have green leaves. But certain types of *S subglabrescens* particularly the rain-fed *Sen cholam* have light green leaves. Dark green is the prevalent colour in the African group *Caffra*. In crosses between the three grades of green, dark green has proved a monogenic dominant to green, and green a monogenic dominant to light green. A 9 6 1 ratio of dark green, green and light green has been obtained connoting supplementary factors

Summary

A brief review of the three important species of the *Durra* group, viz, *S durra*, *S cernuum* and *S subglabrescens*, with special reference to the Indian and particularly to Madras varieties, is given. *S rigidum*, also included in this group but which does not fit in with this, is omitted. Detailed descriptions and distinguishing characteristics of the varieties of these three species cultivated in the Madras Presidency are provided. A short review of the important characters met with in this group has been added at the end with special reference to panicle shape and glume wrinkling. Figs 1–6 illustrate the different types of panicles, panicle branches, spikelets, grain shapes, glume wrinkling and node hairiness in the different species

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AGE AND AFFINITIES OF THE BAGH FAUNA

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In spite of the varied interest—stratigraphical, palæontological and palæo-geographical, attaching to this series of strata, no adequate attention was paid to their fauna until the present writer began his work   The author has in a series of contributions to the palæontology of these beds,[1] presented the results of his study of the Echinoidea, Brachiopoda, Bryozoa, Lamelli-branchia and the Ammonoidea, and discussed the age of these beds on the basis of the affinities of each fossil group independently of the other, so that we possess satisfactory knowledge of all those members of the Bagh fauna which could be studied with any exactness   Thus it is now that we are in a position to discuss adequately the age of this series of strata and their alleged faunal affinities with the Cretaceous deposits of the Trichinopoly District

Among the earlier workers Carter[2] had assigned a Neocomian age to these beds, while according to Bose[3] these strata stretched over a long period extending from the Albian to the Senonian   The conclusions arrived at by both these workers were, however, based on admittedly tentative identifications of the fossils and as such their views carry little weight

Duncan,[4] who for the first time studied in detail the echinoids from these beds, because of the presence of

Nucleolites similis d'Orbigny

Salenia fraasi Cotteau

Cyphosoma cenomanense Cotteau

Hemiaster cenomanensis Cotteau

and *H similis* d'Orbigny

of which the first species is from the Chloritic Marl and the remaining four from the Cenomanian of Europe, and Lebanon thought it justified to assign to the Bagh Beds an upper Green Sand horizon   This conclusion was roughly corroborated by Vredenburg[5] from his study of the Bagh ammo-

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[1] Chiplonker, 1937, pp 60–71, 1938, pp 300–16, 1939 a, pp 236–46, 1939 b, pp 98–109; 1939 c, pp 255–74, 1941, pp 271–76.

[2] Carter, 1857, pp 621–23

[3] Bose, 1884, pp 37–44

[4] Duncan, 1865, pp 349–63, 1887, pp 81–92.

[5] Vredenburg, 1907, pp 109–25, 1908, pp 239–40

nites; while, according to Fourteau[6] both the echinoids and the ammonites point to a lower Gault horizon.

More recently Mukerjee,[7] who was working on a small collection of Mollusca from a few exposures of the Bagh Beds in the Jhabua and Ali Rajpur States, has like Bose assigned to these beds a long period extending from the Cenomanian to the Senonian, thus regarding them as approximate equivalents of the Cretaceous Series of Southern India. Besides basing his conclusions on extremely inadequate palæontological evidence, as the present writer has shown it to be in one of his earlier contributions to the palæontology of the Bagh Beds, Mukerjee curiously enough thinks it reasonably possible to compress the major portion of the Upper Cretaceous Period, from the Cenomanian to the Senonian, in these poorly fossiliferous limestones attaining a thickness of hardly forty feet as they do in Jhabua and Ali Rajpur States

To consider then from the present writer's work such of the more important features of the palæontological evidence as will help us to determine the age of the Bagh fauna as a whole, we find that out of the total of forty-nine species which the author has recorded from these deposits, we have only five species,

Neithea morrisi Pictet and Renevier
Plicatula batnensis Coquand
Hemiaster heberti (Coquand)
H saadense Peron and Gauthier
and *H meslei* Peron and Gauthier

which are known to occur outside the Narbada valley[8] Of these, the first is a very common species in the various sections in the type area around Chirakhan (lat. 22° 22′ 30″, long 75° 7′ 30″), and occurs in the Albian and Aptian beds in England, Spain, Switzerland and Japan, while the other four species are recorded from the Cenomanian beds in Algeria, Tunis and Egypt

Six, out of the seven species of *Hemiaster* represented in these beds[9] belong to the groups of *Mecaster, Proraster* and *Integraster*, all of which make their first appearance in the Cenomanian Further, we have in these deposits the genus *Diplopodia* which is not known to survive the Cenomanian age, while *Hemiaster fourteaui* Chiplonker, the commonest of the Bagh species, has its nearest ally *H luynesi* Cotteau in the Cenomanian of Palestine

[6] Fourteau, 1918, pp 34–53
[7] Mukerjee, 1935, p 73 , 1936, p 81 , 1938, pp 193–98
[8] Chiplonker, 1937, pp, 65, 67, 1939 a, pp 241–42, 1939 c, pp 258, 260
[9] Chiplonker, 1937, pp 62–67, 1939 a, pp 240–44

Among the remaining echinoids, while there is a mixture of lower and upper Cretaceous affinities, the majority present an unquestionable lower Cretaceous aspect. Thus the echinoids on the whole point to a Cenomanian (probably the lower portion, as is shown already) age. Among the ammonites[10] which are represented by three species, *Namadoceras scindia* Vredenburg has probably Turonian affinities, but *Knemiceras mistoi* (Vredenburg), the commonest of the ammonites, has an aspect a little younger than the Vraconian; while *Namadoceras boaei* Vredenburg has distinct double Cenomanian affinities. The ammonites, therefore, on the whole point to a middle to upper Cenomanian age. The brachiopod genus *Mahrihynchia*[11] which features very conspicuously in the type area for the Bagh Beds, has unmistakable Upper Green Sand affinities. The Lamellibranchia and the Bryozoa, though, as is already remarked, show a mixture of affinities ranging over a considerable part of the Cretaceous period, they, particularly the Lamellibranchia, indicate middle Cretaceous as the predominant phase in their affinities.[12]

Thus, while discussing the age of the Bagh Beds on the basis of their fauna as a whole, we are faced with a certain amount of diversity of evidence as furnished by the various groups of fossils. It is, however, not an uncommon occurrence, because all groups of animals inhabiting a particular basin of sedimentation do not necessarily, and often they do not, flourish nor evolve at the same rate as those in the neighbouring basins. But each group of animals is, however, bound to show more or less close affinities to their allies in the adjoining basins, in accordance with the environments as they affected them. Hence the inference of the age of the deposits containing them, when based on the affinities of each group of animals separately, is bound to be more or less different. Therefore, while fixing the age of a formation we have to attach more weight, not to the whole range of affinities shown by all the various forms, as was done by Mukerjee,[13] but to the more predominant elements of the fauna and the general aspect as shown by the assemblage of species.

In the present case we have recorded in these beds a few species of which the age in other areas is definitely known, while for the rest of the species we have to rely upon their affinities towards species in other parts of the world. Thus with the four Cenomanian species mentioned above and the definite Cenomanian aspect of the echinoids, brachiopods and the ammonites which

[10] Chiplonker, 1941, pp 271–75

[11] Chiplonker, 1938, pp 312–13

[12] Chiplonker, 1939 *b*, pp 99–106, 1939 *c*, pp 256–70

[13] Mukerjee, 1938, pp. 197–98.

form the more dominant members of the Bagh fauna, we are justified in considering Cenomanian as the most appropriate age for these beds

In the list of the Bagh fossils Bose mentions a number of South Indian Cretaceous species   While admitting that they were only roughly identified, he endeavours to show with their help that the different members of the Bagh Beds are approximately equivalent to those of the South Indian Cretaceous Series[14]; and to explain what he considers as anomalous occurrences of some of the South Indian species in the Narbada valley, he[15] invokes the idea of submergence of a land barrier intervening between the Narbada valley and the Trichinopoly District, during the "Nodular Limestone" period, which could thus facilitate the intermigration of the faunas of these two zoological provinces   Bose's work has already been sufficiently criticised by Duncan,[16] and any further allusion to even the more important features of his work would be nothing but repetition of Duncan's remarks

The present author had an opportunity of seeing Mukerjee's molluscan collection from the Jhabua-Ali-Rajpur area, it is neither extensive nor well preserved, and as mentioned on a previous occasion,[17] it needs a closer study before his claim for the presence of *Protocardium pondicherriense* d'Orbigny and *Cardium (Trachicardium) incomptum* Sowerby in the Bagh Beds could be accepted   *Turritella (Zaria) mulitstriata* Reuss is another species in Mukerjee's collection, which he[18] mentions as a typical South Indian form   It is neither a characteristic fossil in the Trichinopoly deposits nor is it in any way typical of South India   It is reported to be quite widely distributed in Lybia and Central Europe   This species, however, as judged from the numerous published figures and descriptions, appears to be a heterogeneous group of, in all probability, related forms, and the specimens from the Bagh Beds of Jhabua identified by Mukerjee as *Zaria mulitstriata* Reuss, might be found to belong to this stock from Central Europe   During the course of the writer's study of the Bagh fossils *Pinna mathuri* Chiplonker,[19] is found to be the only species in his collection which shows some distant relations to *P arata* from South India and *P vanhepeni* from Pondoland, of which the latter two species are again allied to European stock   These species therefore, cannot be considered as presenting a South Indian element in the Bagh fauna; they rather add to the already abundant evidence which the present author has brought forth from his study of all the different groups of fossils

[14] Bose, 1884, pp 37-43, 48-50, Oldham, 1893, p 250
[15] Bose, 1884, pp. 38-39.
[16] Duncan, 1887, pp. 81-92.
[17] Chiplonker, 1939 c, p. 271.
[18] Mukerjee, 1938, pp 187-98.
[19] Chiplonker, 1939 c, pp. 256, 270-71.

from the Bagh Beds, to show that, the fossil fauna of the Narbada valley belongs to the Mediterranean zoological province and had no direct connection across the Indian Peninsula, with that of the Southern Ocean.

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ON THE INTERNAL BUNDLES IN THE STEM OF
RUMEX PATIENTIA L.

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1. *Introduction and Previous Work*

A few years ago, one of us (Maheshwari, 1929) investigated the anatomy of *Rumex crispus* and found that the inflorescence axis of this plant possesses a number of internal and inverted bundles in addition to the normal outer ring of vascular bundles. A detailed study of their origin and development revealed that they are not formed by the division of pith cells as was previously supposed (Hérail, 1885) but from the inner portions of the same procambial strands which produce the normal bundles. It was also demonstrated that the bundles are not merely inverted or obcollateral but actually become concentric (amphivasal) due to the extension of the cambium all round the phloem. The xylem in the internal bundles is entirely secondary in origin, while the phloem is both primary and secondary. A maximum of five internal bundles was found in association with a single normal vascular bundle.

Since then Joshi (1931), in a brief note on the anatomy of *Rumex dentatus*, mentions that while the stem is normal in structure and devoid of any internal bundles, there arises occasionally in the basal internodes a pericyclic cambium, which forms an accessory ring of bundles between the cortex and the primary vascular ring.

Würke (1933), in a work dealing with the anatomy of the rhizome of *Rheum*, also makes some casual remarks on the anatomy of a few species of *Rumex R. patientia*,[1] *R crispus* and *R domesticus* were found to have bicollateral bundles, whose internal phloems later on give rise to inverted bundles. Further details are not mentioned and after a general discussion and confirmation of Maheshwari's work on *R. crispus*, the author passes on to *Rheum*, which forms the main part of his contribution.

2 *Observations*

The material used in the present study was collected by one of us[2] in 1936, from the suburbs of Kiel, during an excursion with Prof. G. Tischler, and consisted of a few pieces of the inflorescence axis preserved in formalin-

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[1] According to Hérail the internal bundles of *R patientia* consist of phloem only

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acetic-alcohol. Sections were cut freehand and stained in Safranin and Fast
Green.

A cross-section of an internode shows a hollow pith and a single ring
of vascular bundles which at first appear to be quite normal A closer exami-
nation reveals, however, the presence of internal phloem in many of the
bundles which are thus truly bicollateral The external surface is provided
with ridges and furrows, with collenchyma occurring underneath the former
(Fig 1)

Sections of the older internodes presented a still larger number of bi-
collateral bundles, the internal phloem lying completely within the sclerenchy-
matous sheath of the outer bundle (Fig 2) and not scattered in patches at the
periphery of the pith as is the case in the Convolvulaceæ, Solanaceæ, Apocy-
naceæ, Asclepiadaceæ, etc   The older of the phloem groups showed a
distinct cambium on their outer side, just facing the protoxylem (Fig. 5).
This cambium has a clear tendency towards lateral extension and later
completely surrounds the phloem (cf *Rumex crispus*)   Most of its activity
is however confined to the outer side only, resulting in the formation of

obcollateral or inverted bundles The xylem in this bundle is obviously all secondary but the phloem is partly primary and partly secondary The central region of the phloem, which is primary, is particularly rich in sieve tubes. In a few cases the internal bundles were ob-bicollateral with phloem in the centre and xylem on both sides (Fig. 4) or amphivasal (Fig 3) with xylem vessels or xylem parenchyma completely surrounding the phloem.
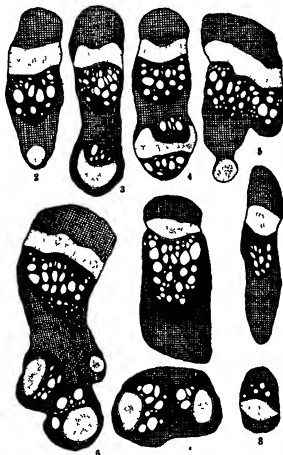
FIGS. 2–8 Figs 2–6 Vascular bundles of the inflorescence axis of *Rumex patientia* showing various stages in the development of internal bundles. × 173 Fig. 7 A normal bundle of *Rumex crispus* with two adjacent internal bundles × 173 Fig. 8 A normal and an internal bundle of *Rheum rhaponticum* L × 173

The oldest pieces of the stem, available to us, showed conspicuous internal bundles. In some sections, 3 such bundles were found to be associated with a single outer bundle, all enclosed in a common sheath of sclerenchyma and forming a distinct unit (Fig. 6) In rare cases a single internal bundle lies in association with 2 partially anastomosed normal bundles (Fig 5).

For comparison, we examined some previously prepared slides of *Rumex crispus* and found that there is no essential difference in the stem anatomy of the two species. In one case, in *R crispus*, we found two internal bundles detached from the sheath and lying at some distance inward in the pith (Fig. 7) Their deeper position does not mean, however, that such bundles actually arise through a division of pith cells. It is more likely that a sclerification of the cells between the outer and inner bundles was probably delayed for some time and when it did take place, each side formed its own sheath, leaving some unlignified parenchyma between

Such a separation of the internal bundles which is rare in *Rumex*, is common in the allied genus *Rheum* (Fig. 8) According to Würke the conditions in the two genera are closely similar in the earlier stages but later on the internal bundles of *Rheum* get detached from the normal vascular ring due to a division of the intervening cells

We do not deny the possibility of the formation of internal bundles from pith cells in other plants. Indeed, this has been conclusively demonstrated in the case of tobacco (Esau, 1938), where pith cells resume a meristematic character and give rise to groups of sieve tubes irregularly arranged in the perimedullary zone.

3. Conclusion

The phylogenetic or physiological significance of the internal bundle has been discussed by several authors. Worsdell (1915, 1919) considers the medullary phloem to represent a vestigial structure, the remnant of a former system of medullary vascular bundles, in which the xylem has disappeared and adds that " the morphological origin of this internal phloem bundle is from an amphivasal bundle, for the latter is the typical and more primitive form of the medullary phloem bundles, wherever they occur ". The inversely oriented internal bundles are explained by supposing that only the outer portion of the originally amphivasal bundle is retained.

Maheshwari (1929), in his study of *Rumex*, adduced evidence to show that the presence of internal bundles is an advanced character, the species with higher chromosomes being generally found to possess them and those with the lower numbers lacking them. Würke (1933) is in complete agreement with this view Alexandrov and Alexandrova (1926) and Hartwich

(1936), working on the internal bundles of the inflorescence axis of *Ricinus communis*, also regard their presence as derived.

In order to test the hypothesis that the species of *Rumex* with higher chromosomes are likely to be provided with internal bundles while those with lower numbers are likely to be without them, it is necessary to examine more material belonging to a variety of forms. With an instrument like colchicine in hand, it may also be possible to induce polyploidy either in the same species or its hybrids and then examine them anatomically to see if this change is associated with the appearance of the internal bundles. This would open up a new field of experimental anatomy, that would probably lead to the solution of other problems

4 Summary

The inflorescence axis of *Rumex patientia* has bicollateral bundles whose internal phloems give rise to obcollateral, ob-bicollateral and amphivasal bundles Sometimes two or three internal bundles lie in association with a single outer bundle It is concluded that the presence of such bundles is an advanced character Further, the condition in *Rheum*, where the internal bundles lie detached from the outer ones, is easily derived from that in *Rumex*.

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THE EMBRYO-SAC OF *EUPHORBIA HETEROPHYLLA* L.—A REINVESTIGATION

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I Introduction and Previous Work

FROM the point of view of floral morphology and embryology, the family Euphorbiaceæ has been of great interest for a long time No less than 8 different types of embryo-sacs have been reported which are shown diagrammatically in Fig. 1 Most of the plants exhibit the *Normal*-type with a monosporic, 8-nucleate gametophyte Modlewski[1] (1909, 1910, 1911) found a tetrasporic, 16-nucleate type in *Euphorbia procera* and *E palustris*, which is now designated as the *Penæa*-form and has since been reported in *Acalypha australis* (Tateishi, 1927) In *Mallotus japonicus* (Ventura, 1934) also, 16 nuclei are present but they are organised differently—an egg apparatus, two polar nuclei and eleven antipodal cells (*Drusa*-form) Arnoldi (1912) thought that he had found 4-nucleate embryo-sacs developing from a single megaspore (*Oenothera*-type) in *Glochidion*, *Codiæum* and *Ceramanthus* (*Phyllanthus*) This claim was disproven in the case of the last two genera by Lundberg (1931) and Maheshwari and Chowdry (1936) respectively and it seems that *Glochidion* will also yield similar results on reinvestigation It is certain that Arnoldi missed the antipodal cells in his preparations and was thus led to a wrong interpretation (for a detailed discussion see Maheshwari, 1937).

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[1] In his "Introduction" Sanchez (1938) quotes Modilewski's work (1910) on *Euphorbia procera* as follows :—

> "In this species, instead of only one surviving megaspore dividing to form the normal 8-nucleate embryo-sac, all the four megaspores divide simultaneously without exception so that each of them after two successive divisions gives rise to a 4-nucleate embryo-sac. At this stage, one of the 4-nucleate embryo-sacs divides twice to form a mature 16-nucleate embryo-sac, while the other three degenerate"

This is obviously a misunderstanding of the original in German What Modilewski really says, is that there are several megaspore-mother cells, all of which may undergo reduction division and become 4-nucleate Only one divides further, however, and forms a 16-nucleate embryo-sac. He summarises the situation as follows (p 417) :—

> "Eine von den viernaligen Embryosackmutterzellen entwickelt sich zu einem reifen sechzehnkernigen Embryosack, während die übrigen degenerieren."

Type	Megaspore mother cell	I Division	II Division	III Division	IV Division	V Division	Mature embryo sac	Remarks
Normal-type								Occurs in by far the largest number of investigated genera and species.
Oenothera-type								Reported in *Codiaeum, Ceramanthus* and *Glochidion* by Arnoldi (1912). First two cases already disproved (see Nakashwari, 1937); the third is also extremely doubtful.
Allium-type								Reported in —*Euphorbia mauritanica* (Venkata, 1935).
Penaea-form								Reported in —*Euphorbia procera* (Modlewski, 1909) *E. palustris* (Modlewski, 1911)
Acalypha indica-form								Reported in *Acalypha indica* (See Shashukari and Johri, 1910)
Fritillaria-type								Probably occurs in *Euphorbia dulcis* (Carano, 1926)
Drusa-form								Reported in *Mallotus japonicus* (Venkata, 1934)
Adoxa-type								Reported in *Euphorbia heterophylla* (Kamikaa, 1936) Dealt with in the present paper and shown to be incorrect.

Fig. 1. Diagram showing the different types of embryo-sac development reported in the Euphorbiaceae

Carano (1926) noted that in *Euphorbia dulcis* three of the four nuclei, formed after the first two divisions, pass down to the chalazal end of embryo-sac and during the next division the spindles of these 3 chalazal nuclei fuse together resulting in a secondary 4-nucleate stage. This by a further division gives rise to an 8-nucleate embryo-sac organised in the normal fashion A comparison with Bambacioni's figures of *Fritillaria persica* (1928) seems to indicate that the development is identical.

Yet another variation was noted by Ventura (1933), who reports an *Allium*-type of embryo-sac in *E mauritanica*, and D'Amato[a] (1939), in a work dealing with several spp of *Euphorbia*, reports that in most cases the *Normal*-type was observed, but some spp occasionally or regularly show the *Allium*-type

More recently Maheshwari and Johri (1940) have published a prelimi-nary note on the embryo-sac of *Acalypha indica*, in which 16 nuclei, formed by 2 divisions of the megaspores, are sometimes organised as in *Euphorbia virgata* and *Acalypha australis*, but more frequently form four pairs of 2 cells each leaving 8 nuclei to fuse in the centre. Several other irregularities have been noted and described in the full paper which is in the press.[b]

The embryo-sac of *Euphorbia heterophylla*, which forms the subject of the present paper, was first investigated by Modilewski (1910), who reported a *Normal*-type of embryo-sac in this and several other species of the same genus His description of this species being very brief and unillustrated, Sanchez (1938) reinvestigated it, and found an *Adoxa*-type of embryo-sac From his figures and descriptions, however, this appeared so doubtful that a reinvestigation was undertaken.

2 *Observations*

The material was collected from some plants growing in the Government Nursery, Dacca, and in the writer's private garden Nawaschin's fluid and formalin-acetic-alcohol were used for fixation The sections were cut at $10\,\mu$ and stained in iron-hæ matoxylin

A description of the ovary and ovules has already been given by Sanchez and my observations agree with his. The growth of the integuments is very tardy but the nucellus is well developed The inner integument starts first at about the time the megaspore-mother cell is already well formed, but is soon surpassed by the outer

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[a] The full paper of this author was not available and hence I am unable to discuss his observations in detail.

[b] This was to have been published a couple of years ago in the *Beih bot Cbl*, but no information about it is now available due to the war.

With regard to the early development of the embryo-sac Sanchez writes as follows:

"Following the synezetic contraction the macrospore-mother cell proceeds to usual heterotypic division, resulting in the formation of two daughter nuclei. These nuclei move and lie opposite each other at the ends of the enlarged macrospore-mother cell (Fig 3 B)[a] one toward the micropylar end and the other toward the chalazal end. A large vacuole is formed between them while the two daughter nuclei become surrounded with dense protoplasm. From this stage the macrospore-mother cell grows rapidly especially in width (Fig. 3 B).

The two daughter nuclei divide simultaneously in the usual manner into 4-nuclei. These nuclei are not separated by walls, but remain in pairs separated by the large central vacuole. All the four macrospores become functional because they all participate in the formation of the female gametophyte or embryo-sac. Thus none of them degenerated or disintegrated as observed in the normal type of embryo-sac development, where only one surviving macrospore becomes functional. The third division seems to take place immediately after the four nuclei are fully developed, for the 4-nucleate embryo-sac stages are comparatively few."

A critical study of the figures presented by Sanchez, the more important of which are reproduced here (Fig 2) reveal a great gap between the megaspore-mother cell and the 2-nucleate stage (compare also his diagrams of the ovules at this stage, Fig. 2 *a-c*). The beginning of vacuolation even before the reduction divisions are over is most unlikely. As pointed out by Rutgers (1923), Fagerlind (1938) and Maheshwari (1941), polarity in the embryo sac begins only after the formation of the megaspores. There is no case where it is known to commence immediately after the first reduction division as supposed by Sanchez.

Indeed it was this discrepancy in the drawings of Sanchez that suggested this reinvestigation.

My observations show that the megaspore-mother cell stage is identical with that figured by Sanchez (Figs 3 and 4). The first reduction division is followed by wall formation and the second division also proceeds quite normally in the lower dyad but is frequently delayed in the upper. A row of four cells is formed in most cases but sometimes the division is incomplete in the upper dyad and only 3 cells are then observed (Figs 5–8).
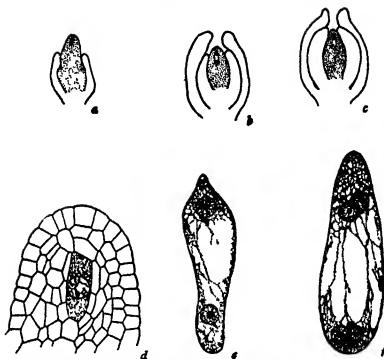
[a] This is reproduced here as Fig. 2 *e*

Fig 2. Drawings of megaspore-mother cell and 2-nucleate and 4-nucleate embryo-sacs (copied from Sanchez, 1935) Figs *a*, *b* and *c* show diagrams of ovules at the stages shown in *d*, *e* and *f*

The lowest cell of the tetrad enlarges and functions I was able to see the 3 degenerating megaspores quite distinctly in several preparations and can therefore state definitely that the development is not of the Adoxa-type but of the Normal-type

Vacuolation begins first only after megaspore formation has been completed and the chalazal cell has enlarged appreciably The subsequent stages showing 2, 4 and 8 nuclei are passed through normally and are identical with those figured by Sanchez. It is therefore unnecessary to duplicate them here.

3. Discussion

The above observations remind one of exactly similar errors committed by some other authors Perhaps, *Typha latifolia* presents a more or less

identical case. Schaffner (1897) studied the embryo-sac of this plant and mentions having taken extreme care in tracing out the development step by step A row of megaspores was "never seen" and the development was stated to be of the *Adoxa*-type Nevertheless, Dahlgren (1918), twenty years later, proved that the 4 cells are formed as usual and it is the chalazal megaspore which functions to give rise to the embryo-sac.
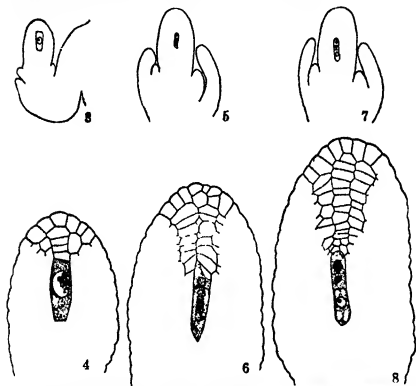

Fig 3 L.S ovule at megaspore-mother cell stage × 300 Fig 4 Nucellus with megaspore-mother cell × 1,050 Figs. 5, 7 L.S ovule with stages in megasporogenesis × 300 Figs. 6, 8 Nucelli of ovules in Figs. 5 and 7 respectively × 1,050

It is a great pity that several authors have not drawn any detailed drawings but merely given diagrams showing the number of nuclei. It is impossible to discuss these cases, but other errors of interpretation, where at least the illustrations were executed with sufficient care, have often been

rectified without much difficulty The chief traps for workers on embryo-
sac development are either in connection with megasporogenesis or the
organisation of the mature embryo-sac With regard to the former it may be
stated that as a rule *vacuolation and polarity in the embryo-sac follow the forma-
tion of megaspores and are never seen before the reduction divisions are over*
Thus, the megaspore-mother cell and dyad stages are free from any conspi-
cuous vacuoles The early tetrad stage also does not show appreciable
vacuolation, which starts first only after the four megaspores (or megaspore
nuclei) have been formed and the next stage is about to commence. This
rule applies to all embryo-sacs, whether monosporic, bisporic or tetrasporic
Thus, the difference between the four-nucleate stage of a monosporic embryo-
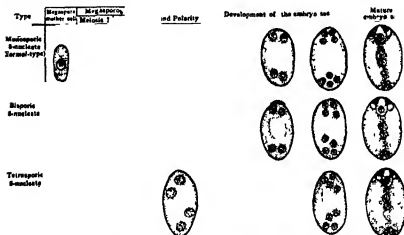sac and a tetrasporic one is very well marked (Fig. 9). The former shows

FIG 9 Diagram to show the stage at which vacuolation begins in the development of
monosporic, trisporic and tetrasporic embryo-sacs

a large central vacuole with the nuclei and cytoplasm limited to the periphery,
while the latter begins to show a central vacuolation only at a later stage,
just preparatory to the next division, and even then this is far less appreciable
than in the monosporic and bisporic embryo-sacs Note also the difference
between the 2-nucleate stage of a monosporic and a tetrasporic embryo-sac
(Fig. 9). The well-marked polarity and vacuolation in Figs. 3 b and 4 a
of Sanchez (reproduced here as Figs 2 e and 2 f) entirely go against the
interpretation that they are pre-reduction stages. ✓

I should like to state here that I do not consider vacuolation and polarity to be an infallible means of judging whether the embryo-sac is monosporic or tetrasporic, but I agree with Rutgers (1923) that it serves as a good and fairly reliable guide, which merits proper consideration

The other error, concerning the stage immediately preceding the organisation of the mature embryo-sac, is also well exemplified in the Euphorbiaceæ Arnoldi (1921) mistook the normal 8-nucleate embryo-sacs of *Codiæum* and *Phyllanthus* for 4-nucleate because of the early fusion of the polar nuclei and the disappearance of the antipodals Sometimes the antipodal cells (or nuclei) may be in a narrow porch that is easily missed in thin sections In still other cases they become laterally placed due to a downward extension of the main body of the embryo-sac (see Kajale, 1940, and the literature quoted therein)

4 Summary

A reinvestigation of the embryo-sac of *Euphorbia heterophylla* shows that the development is not of the *Adoxa*-type as reported by Sanchez (1938) but of the *Normal*-type The megaspore-mother cell gives rise to a tetrad of megaspores of which the chalazal cell functions to produce a normal 8-nucleate embryo-sac

It is pointed out that as a rule vacuolation and polarity are not seen in embryo-sacs until after the reduction divisions are over They form such reliable guides that all cases, where the reported observations are not in accordance with the above, deserve a fresh study

5 Acknowledgements

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A LEAF SPOT DISEASE OF ZINGIBER OFFICINALE CAUSED BY *PHYLLOSTICTA ZINGIBERI* N.SP

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IN 1938 and succeeding years a leaf spot disease has been reported on ginger from Godavari and Malabar districts   The disease is common in the months of August, September and October   The spots vary in size   Some are small and roundish being a millimeter in length and half in breadth   Others are oval or elongated having a size of 9–10 × 3–4 mm (Fig. 1)   The spots are almost white in the centre and have a dark brown margin   Just surrounding the spot is a halo of yellowish colour   The central portion is thin and papery and more often torn up   In this portion are also seen a number of minute blackish pycnidia   The pycnidia are formed immersed in the tissues of the leaf under the epidermis   But later they become erumpent and can be seen distinctly on the surface as the mesophyll tissue collapses and the leaf becomes thin in the affected areas   The spots are usually isolated but they may also become confluent resulting in big patches   Sometimes a large number of spots develop on a leaf and in consequence the entire leaf turns brown and dries up

Microscopic examination revealed that the pycnidia are those of *Phyllosticta*   Each pycnidium measures 78–150 μ in diameter and has a definite ostiole   When mounted on slide the characteristic worm-like mass of spores coming out of the ostiole can be seen under the microscope   The spores are hyaline, oblong and measure on an average 4 3 × 1 6 μ the range being 3 7 — 7·4 × 1 2–2 5 μ

Other leaf spot diseases have been recorded on ginger   Sundararaman (1922) has described *Colletotrichum zingiberes* as the cause of a leaf spot disease in Godavari district of the Madras presidency   Stevens and Atienza (1932) have reported from the Philippines a leaf spot of ginger caused by *Coniothyrium zingiberi*   In the description of the fungus they have stated that it may be mistaken for a *Phyllosticta*   The same has been observed in Hawaii (1937)

Examination of the specimens from Godavari and Malabar districts showed only *Phyllosticta* on the spots   The fungus was readily brought into

culture by transferring bits of affected portions of leaves to french bean agar plates after having previously sterilised them by immersion in mercuric chloride solution (1/1000 strength) for 2 minutes and washing in sterile water   In a week's time pure growths developed and began to form pycnidia on agar   From these further isolations were made

The fungus grows readily on agar media. The following statement gives the growth characters of the fungus on the different media tried

TABLE I

Growths on Different Media

Medium	Nature of growth
French bean agar	Thin, greyish white aerial growth, pycnidia in plenty on the medium or slightly immersed, zones faintly visible
Quaker oats agar	Thick growth, aerial growth white, but submerged portion dark olive, pycnidia in plenty but hidden by the aerial mycelium
Potato dextrose agar	Thick growth, aerial growth smoke grey, submerged growth dark olive, zones visible, pycnidia numerous
Richards' agar	Thick growth, aerial mycelium creamy white, submerged growth dark, margin irregular, pycnidia formed
Sterilised ginger leaves	No aerial growth but entire leaves studded with numerous pycnidia

On culture media the pycnidial formation starts on the 4th or 5th day   The pycnidia are light in colour in the beginning but with age the colour deepens and finally they turn light to deep brown   They are isolated or in groups   Each pycnidium has an ostiole and a very short neck   Sometimes a pycnidium shows two ostioles (Fig 4) in all probability formed by the fusion of two pycnidia   The wall of the pycnidium is thin   The pycnidia formed in cultures are much bigger than those in nature   Some are spherical but in most cases they are only subglobose   They measure on an average 177 6 μ (range 100–270 μ)   The ratio of the two diameters is 1 1·1

The hyphæ are hyaline or coloured   Sometimes several hyphæ unite to form strands   Coloured hyphæ are common on potato dextrose, quaker oats and Richards' agars   On french bean agar and sterilised ginger leaves coloured hyphæ are rare   The hyphæ very often form swollen cells of various shapes.   Round glistening bodies are found inside these and some coloured hyphæ (Fig. 6)

The spores are hyaline and oblong with rounded ends   They are often biguttulate (Fig. 5)   Even after keeping in culture for over three years no coloured spore was ever noticed in any of the cultures

The fungus grows well over a wide range of H-ion concentration. It was grown on Richards' agar of different pH values and the diameters of growths are represented below

TABLE II

Growth on Media of Different pH Values

pH value of media	3 5	4 3	4 9	5 8	6 7
Diameter in mm in 7 days	50	63 5	62	62 5	56

The best growth is formed between 4 3 and 5 8 with a falling off on both sides of this range

Pathogenicity—The parasitism of this fungus was tested by inoculations on the leaves of ginger plants When bits of culture and spore suspensions from growths on agar media were used, successful infections were obtained only when the leaves were previously wounded The controls and inoculations made on unwounded surfaces remained healthy without developing any pathological symptoms But inoculations on wounded leaves produced small water-soaked spots on the third day Later there was an increase in size of the spots The central portions of these spots became yellowish and thin and still later dried into white membranous patches showing tearing of the tissues (Fig 2) Pycnidia developed in the central portions in 8 days

Wound infections were successful on the leaves of turmeric (*Curcuma longa*) Spots with white thin membranous centres developed and in these pycnidia were formed

Inoculations were made on ginger leaves using cultures grown on sterilised ginger leaves Two series of experiments were conducted, one within 6 months of the isolation of the fungus and another after two years In the earlier inoculation experiments successful infection was obtained on unwounded ginger leaves but it took a longer time for the spots to develop On wounded leaves evidences of infection were noticed in 60 hours but on unwounded leaves these were visible only after 6 days In the second series of experiments an isolate which had been for two years in culture was grown on sterilised ginger leaves and spore suspensions from this were used for inoculation purposes But no successful infection was obtained when the leaf surface was free from wounds The parasitism of the old culture was not improved by one passage through sterilised portions of the host tissue In nature injuries caused by insects might help in easy infection of leaves.

The symptoms of the disease agree with those described by Stevens and Atienza (1932) But the fungus under study is undoubtedly a *Phyllosticta* whereas the fungus responsible for the disease in the Philippines is described as a *Coniothyrium* though the authors state that it may be mistaken for *Phyllosticta* The local isolate did not show any coloured spores though it has been under observation for over three years Stevens and Atienza give the range of spore size as 3 5–4 × 7–10μ but the average is not given The average of 200 measurements of the spores of the local isolate is 1 6×4·3μ the range being 1 2–2 5×3 7–7 4μ This is decidedly much less than that recorded for the Philippine organism For these reasons and since no *Phyllosticta* has been recorded on ginger till now the local organism is named *Phyllosticta zingiberi*

The diseased plants were obtained from the same village where Sundararaman (1922) had first noticed leaf spot caused by *Colletotrichum zingiberea* But this fungus was not noticed on any of the specimens

Control—At present this is not a very serious disease of ginger But it has been observed to be common in Godavari and Malabar districts and in some years causes a reduction in yield of rhizomes due to the destruction of large areas of chlorophyllous tissue Preventive measures have been carried out against this disease with success in Godavari district The plants are sprayed with 1% Bordeaux mixture before the outbreak of the disease and once again if necessary and these operations are reported to have given good protection against infection

Phyllosticta zingiberi.—Spots oval or elongated, centre whitish, pycnidia on both sides of the spot, subglobose, dark brown in colour, ostiolate, pycnidia from infected plants 78–150μ in diameter, spores hyaline one-celled, oblong, 4 3 × 1 6μ, (3 7–7 4 × 1 2–2 5) biguttulate. On culture media pycnidia generally larger

Habitat—In spots on the leaves of *Zingiber officinale*

Phyllosticta zingiberi—Maculæ ovales vel elongatæ, centro subalbidæ; pycnidiis in utraque superficie maculæ, subglobosis, coloris fuscis, ostiolatis; pycnidiis plantarum infectarum diametro 78–150μ, (pycnidiis autem medii culturæ generatim latioribus), sporis hyalinis, unicellularibus, oblongis 4 3 × 1 6μ (3 7–7 4 × 1 2–2 5) biguttulatis
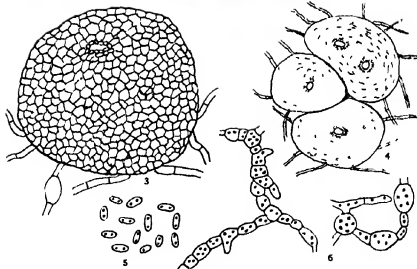
Habitat—Maculæ foliorum *Zingiberi officinalis*

The type specimen is deposited in the herbarium of the Government Mycologist, Agricultural Research Institute, Coimbatore, S. India

Phyllosticta _engberti_ n. sp.

FIG. 1   Diseased leaves from nature   FIG. 4   Group of pycnidia (diagrammatic)
FIG. 2   Spots formed on inoculated leaves   FIG. 5   Spores   600
FIG. 3   A pycnidium from culture   ×200   FIG. 6   Irregularly swollen hyphæ from culture   ×600

I am thankful to Mr K. M. Thomas the Government Mycologist who has helped me in various ways during this investigation I am indebted to Rev Fr Bahan, s.j., of St. Joseph's College, Trichinopoly, for the latin translation of diagnosis

Summary

A leaf spot disease caused by *Phyllosticta zingiberi* is common in Godavari and Malabar districts. Spots with whitish centres develop on the leaves and in these pycnidia of the fungus are formed Wound inoculations were successful on ginger and turmeric Soon after isolation, cultures on ginger leaves are able to infect unwounded ginger leaves.

This fungus does not agree with the description of *Coniothyrium zingiberi* The spores are smaller and never coloured Hence it is given the name of *Phyllosticta zingiberi*.

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THE ORIGIN OF SIPHONOSTELE IN THREE SPECIES OF *SELAGINELLA* SPR.*

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Introduction

SEVERAL types of steles are found in the vascular plants, but in the earliest of these plants, such as, the Psilophytales, the Psilotales, several fossil and living Lycopodiales and a few of the living ferns, particularly in their seedling stages, the axes are characterised by a solid hadrocentric stele This solid xylocentric stele is, therefore, taken to be the primitive stele or the *protostele* from which all the other types of steles are believed to have been derived in the course of phylogenetic specialisation

One of such derivatives is the *siphonostele* with pith or medulla in the centre Two types of siphonostele are met with, the *ectophloic siphonostele* and the *amphiphloic siphonostele* The former has phloem only on the outside of the tubular xylem This is by far the most common type found in the axes of Gymnosperms and Angiosperms[2] and in ferns, like Osmunda The amphiphloic siphonostele, on the other hand, has phloem on both sides of the xylem cylinder and is represented in the living ferns, like *Adiantum, Pteris, Osmunda cinnamomea, Botrychium, Todea hymenophylloides,[1] Ophioglossum vulgatum, Botrychium Lunaria,* etc , and also in some families of herbaceous Angiosperms[2]

So far *three* possible ways of pith formation have been suggested (1) The pith is the included cortex or the fundamental tissue Hence its origin is *extrastelar* (2) The pith represents the undifferentiated xylem Hence the origin is *intrastelar* (3) The pith is partly extrastelar and partly intrastelar in origin, as in the Osmundaceæ and Ophioglossaceæ[2]

According to Van Tieghem (1890), Jeffrey,[2] Gwynne-Vaughan,[2] Tansley,[14] and Boodle,[1] the pith is extrastelar in origin in the rhizomatous solenostelic (= siphonostelic) ferns Jeffrey[2] further stated that *in all cases* the pith *must* be regarded as derivatives of the cortex, *i e* , the origin of pith in all cases is extrastelar.

From his extensive studies of the anatomy of Pteridophytes, both fossil and living, Bower[2] generalises that the origin of the medullation is determined by two factors, *viz*, the position of axes and insertion of the appendages He concludes that (1) in all upright columner microphyllous stems the pith is tracheary in origin, *i e*, intrastelar, and he cites Lepidodendreæ and the cone of living *Selaginella spinulosa* as examples, (2) in all creeping megaphyllous shoots, *e g*, the rhizomatous ferns, the pith is extrastelar in origin (p 562) Eames and MacDaniels[3], however, think that it has been established beyond doubt that in seed plants at least the pith is morphologically extrastelar; in most of the Pteridophytes the same condition obtains, probably in a few the pith is intrastelar in nature (p 114)

Thus there is still a divergence of opinion as to the origin of pith in the microphyllous upright stem of the Pteridophytes A great difference of opinion also prevails with regard to the origin of the internal phloem and internal endodermis of the amphiphloic siphonostele It will, therefore, be interesting to follow the origin of pith in three of the most specialised species of Selaginella with upright microphyllous axes

These are *S inaequalifolia* Spr, *S Wallichii* Spr and *S canaliculata* Baker Specimens of *S inaequalifolia* were procured from the Orchid House of the Royal Botanic Gardens, Calcutta, and those of *S Wallichii* and *S canaliculata* were supplied by a student from his garden house at Dumdum, a suburb of Calcutta All the three species were kindly identified by the Curator of the Herbarium, Royal Botanic Garden, Calcutta The observations are based upon freehand sections cut serially from base upwards of materials preserved in formol-acetic-alcohol and stained in safranin and fast green

Observations

S inaequalifolia (Figs 1-3) is tri-stelic with an accessory stele in the upright stem The accessory stele gradually enlarges and unites with one of the lateral steles to form the siphonostele in the upper region of the stem, and in so doing encloses a mass of extrastelar ground tissue which forms the pith In this case, therefore, the pith is distinctly extrastelar in origin

S Wallichii (Figs 4-7) is also tri-stelic with an accessory stele in the lower region of the upright stem. Here also the accessory stele enlarges and unites with a lateral stele to form the amphiphloic siphonostele In this species almost all the stages in the origin of the siphonostele from separate steles can be traced The pith is clearly extrastelar in origin, and the character of the pith cells are strikingly similar to those of the cortical cells. The similarity in this case is certainly not "merely physiological" as is assumed by the opponents of the theory of the extrastelar origin of pith,

S canaliculata (Figs 8–12) resembles the other two species in having almost the same structure in the upright stem   Here also the siphonostele in the upper region of the stem has been formed by the union of a lateral and the accessory steles, and more or less a complete series in its origin can be followed   The stage shown in Fig. 12 appears to be different from any described above   It seems that a single stele has enlarged and bent in such a way that the two free ends have come to meet with the result that a mass of extrastelar ground tissue has been enclosed   A siphonostele with pith has thus been formed

Discussion

So far as the writer is aware only two cases of medullations in living *Selaginella* have been reported . one in the creeping rhizome of *S lævigata* Baker var *Lyallii* Spr , reported by Gibson[6] where the stelar arrangement very closely resembles that of a typical Filicinean amphiphloic siphonostele with ramular gaps   Bower[5] admitted with Jeffrey[9] that the pith in this case is extrastelar in origin and cites the case as an illustration of his hypothesis that the origin of pith in *creeping microphyllous* form may be extrastelar by adjustment to resemble that in rhizomatous ferns

The other case is the axis of the strobilus of *S spinulosa* A Br reported by Bower,[5] who found pith in all stages of development in the stele in serial sections from base upwards   He regarded this to be a clear case of intra-stelar origin of pith in a stem where there were no ramular or foliar gaps " to provide that continuity with the cortex without which cortical intrusion cannot take place "   But a doubt certainly arose in his mind as to the real nature of this pith when a longitudinal section through the strobilus revealed the tracheidal nature of its cells (elongated), and the presence of nucleus and protoplasm in them   Prof Bower anticipated that " it may be argued that the softer central tissues are merely the result of imperfect development of tracheids and that they would mature into tracheids later," but stated " that the tissue have the appearance of maturity while the condition of the sporangia and of the strobilus as a whole shews that further development is not to be expected "   Convinced thus of the intrastelar origin of pith in the strobilus of *S spinulosa* he cited this case as an illustration of his general hypothesis that the origin of pith in the upright microphyllous stock is always intrastelar.

Mitchell,[11] who also worked out the anatomy of the strobilus in *S spinulosa*, on the other hand, reported that the vascular system of this organ is essentially simpler than it is in the vegetative axis   It has a single vascular chord with typically 8 marginal protoxylem groups   The metaxylem consists of small elements which are frequently not thickened towards the centre

and there may be more or less a well-marked procambial area extending from the tip downwards

It may, therefore, be suggested that the so-called pith which Prof Bower observed in the strobilus of this species, but the presence of which has not been corroborated by other workers in the young or mature, upright or creeping stem, is a collection of undeveloped tracheids or ill-differentiated metaxylem A comparison of the figures given by Bower (Figs 1 and 5, Pl XLVII), Gibson (Fig. 3, Pl IX) and Mitchell (Fig. 9, Pl VIII), strengthens the suggestion made here Other examples of this type are not rare Prof Gwynne-Vaughan in the sporeling of *Osmunda regalis* noticed the presence of parenchymatous cells surrounding xylem tracheids, which he thought "may be undifferentiated cells in the process of differentiation as they still have well-developed nuclei" Bower's figure of the t s of the rhizome of *Ophioglossum reticulatum* points to the possibility of the few parenchymatous cells noticed in the otherwise solid xylem core as being still undifferentiated tracheids The present writer's observation on the structure of the creeping stolons that are annually given off from the base of the erect rhizome of *Nephrolepis exaltata* Schott. shews that the central procambium takes a very long time to differentiate into metaxylem proper

When the above facts are taken into account with the mode of the origin of the medullation in the upright stems of the three species of *Selaginella* described in this paper Prof Bower's general hypothesis of the intrastelar origin of pith in the upright microphyllous forms seems to need revision. It is seen that the medullation here has nothing to do with the ramular or foliar gaps, and the pith is definitely of extrastelar origin The presence of internal phloem and internal endodermis is satisfactorily explained as due to the origin of the amphiphloic siphonostele from the polystelic condition and as a result of fusion of more than one steles One need not conceive of their origin (or presence) as "entirely *de novo*", or by "decurrency through the branch gaps into the pith", or by "involution at the leaf gap," or by "gradual encroachment upon the pith and then by invagination," or that in their origin they "crept round the edges of the branch gaps," or "have subsided into them".

Selaginella belongs to the class Lycopsida where the steles are not characterised by leaf gaps, and there is no provision in their living representatives for secondary growth in their axes The axes of the species under investigation grow erect and sometimes attain a great length To meet the increasing demand for conducting and mechanical tissues the vascular cylinder here enlarges not by the activity of a cambium which is absent, but

by increasing the number of steles which results in the polystelic condition observed in these species   A similar conclusion was previously reached by Scott[13] working on the origin of polystele in dicotyledons. He stated that the need for the enlargement of the vascular system is met by increasing the number of steles rather than the size of a single central cylinder   Bower[4] also developed the same idea of the expansion of the conducting and mechanical tissues in his *Size and Form in Plants*

Conclusion

The observations recorded in the foregoing pages thus warrant the following general conclusion with regard to the origin of the amphiphloic siphonostele in *Selaginella*

The origin of the siphonostele in *Selaginella* is correlated with the polystelic condition and the amphiphloic siphonostele originated as a result of a fusion of a number of separate steles   The polystelic condition probably originated in response to the necessity of increasing the amount of conducting and mechanical tissues in the absence of provision for secondary growth The pith is extrastelar in origin and the presence of internal phloem and internal endodermis is directly due to the origin of the siphonostele from the polystelic condition

My thanks are due to Dr A C Joshi for his kindly going through the MSS and many helpful suggestions which have considerably enhanced the value of the paper

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EXPLANATION OF FIGURES IN PLATES VI AND VII

All figures are photomicrographic representations

FIGS 1–3, Plate VI *S Inaequalifolia* Spr—Transverse sections at different levels of the upright stem. Stages shewing the union and coalescence of the ventral lateral and accessory steles to form the amphiphloic siphonostele with extrastelar pith

FIGS 4–6, Plate VI and FIG 7, Plate VII *S Wallichii* Spr—Same as above The stages are more complete.

FIGS 8–12, Plate VII *S canaliculata* Baker—Same as above Fig 12 shews a stage where the lateral stele alone appears to have formed the siphonostele

THE GENUS *CEPHALOGONIMUS* IN INDIA AND BURMA

By G D BAHLERAO, D Sc, Ph D

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BETWEEN 1930 and 1937 five species of the genus *Cephalogonimus* have been recorded from India *C emydalis* Moghe, 1930, *C magnus* Sinha, 1932, *C gangeticus* Pande, 1932, *C mehri*, Pande, 1932 and *C minutum* Mehra, 1937 Chatterji (1936) recorded *C burmanica* from a tortoise in Burma. Several writers in the past have utilised the relative position of the testes (tandem or oblique), the comparative size of the suckers, the position of the genital pore, the presence or absence of the œsophagus, the length of the intestinal cæca, the extent of the vitellaria and the cirrus sac, the position of the ventral sucker, the nature of the receptaculum seminis and the position of the ovary as criteria for distinguishing one species of the genus from the other Sinha (1932) has also included the terminal part of the excretory bladder in distinguishing the species *C gangeticus* from *C lenoiri* The writer has found in his studies of these characters that the relative position of the testes (tandem or oblique), the shape of the ovary (almost rounded or transversely oval) and the comparative size of the two testes are variable in different individuals of the same species. The comparative sizes of the oral and the ventral sucker appear to be a constant feature in all the speci. mens from *Lissemys punctata* the oral sucker was invariably larger than the ventral sucker The position of the genital pore and the terminations of the intestinal cæca were also quite constant. Unnecessary importance has been attached to the presence or absence of the œsophagus As a matter of fact, a very short œsophagus at least is always present In certain species, how- ever, its presence cannot be detected *in toto* preparations In such cases, this organ can only be seen in a sectionised specimen. On examination of some individuals of the same species the extent of the vitellaria was found to be extremely variable. This has already been pointed out by the writer in 1936 Usually the left vitelline gland is the longer but, in rare instances, the right side gland was found to be the longer. The location of the ventral sucker in the body varies from the anterior fourth to the anterior third The position of the ovary and the termination of the cirrus sac with respect to the ventral sucker are also somewhat variable. The shape of the

receptaculum seminis among the Trematoda is dependent on the number of spermatozoa it contains In regard to the terminal part of the excretory bladder, it may be remarked that this is a generic character and not a specific one. The omission of a reference to this point is not to be regarded as indicating its absence in the species concerned

In the light of the remarks offered above, it is found that the species *C mehri* and *C minutum* are quite distinct and can be separated from all the known species of the genus In regard to *C burmanica*, Chatterji (1936, 85) mentions that the intestinal cæca terminate " a little anterior to the posterior end of body" In the text-figure 3, on p 85, however, the cæca are shown to terminate a little behind the testes and at a distance much in front of the posterior end of the body If the figure, which is very distinct, is taken to be correct representation of the parasite, it more nearly approximates to the species *C europæus* Blazoit, 1910 From this species it can, however, be distinguished by the extent of the vitellaria, the position of the genital pore and by the length or the œsophagus

It has already been pointed out by the writer (Bhalerao, 1936) that the species *C magnus* and *C gangeticus* are identical and that the latter is a synonym of the former It was further pointed out that both these species are synonymous with *C amphiuma* Chandler, 1923 A recent examination of some material at the disposal of the writer has convinced him that *C amphiuma* is quite distinct from *C magnus* as in the former species the intestinal cæca terminate very close to the posterior end of the body, while in the latter they terminate midway between the hinder border of the posterior testis and the posterior end of the body It has previously been remarked that this character is not subject to variations as are some other organs Further, on comparing *C magnus* with the description of *C emydalis* as given by Moghe (1930) and from some material at the disposal of the writer, it is found that in regard to the internal anatomy the two species are quite identical Pande (1932) states that *C gangeticus* (syn of *C magnus*) differs from *C emydalis* in the subterminal position of the oral sucker, the acetabulum being situated more towards the posterior end, in the presence of an œsophagus, in the cirrus sac not being coiled on itself, in the position of the ovary, the form of the receptaculum seminis and in the location of the uterus and the vitellaria. Examination of some material at the disposal of the writer revealed that, in *C emydalis* also, the oral sucker is subterminal, the ventral sucker is situated between the anterior third and fourth of the body, the œsophagus is very small, the cirrus sac usually coils during contraction, the position of the ovary and the extent and disposition of the vitelline

follicles are always variable and the uterus completely fills up the post-testicular region of the body   The only respect in which *C magnus* differs from *C emydalis* is the relatively large size of its body and organs   It is therefore considered that *C magnus* is merely a large variety of *C emydalis* and not a species distinct from the latter

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BIONOMICS AND CONTROL OF *AEOLESTHES HOLOSERICEA* F. (CERAMBYCIDÆ· COLEOPTERA)

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AND

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Aeolesthes holosericea F was collected for the first time by the Director, Imperial Forest School, Dehra Dun, in 1889 from Sal and *Terminalia tomentosa* Stebbing (1914) made some observations on the life-history of this pest between 1901–09 and also described the adult Beeson (1941) recently discussed its life-history, economic importance, food-plants and control Nevertheless, information as to the duration of its various stages, seasonal history and control (when the attack is in progress) has remained singularly meagre and in the present paper an attempt is made to throw light on these points

Distribution

Stebbing (1914) collected it from U P, Oudh, Hyderabad, C P, D I Khan and Ganjam; Gahan from N W India, Bombay, Nilgiris, Ceylon, Assam, Andaman, Nicobar, Siam and Malay Peninsula and Lefroy secured it from Bengal According to Beeson (1914) *A holosericea* is distributed throughout the greater part of the forest of India, " it extends up the submontane valleys of the Himalayas to considerable elevation, occurs in the Indus plains and in theSundarbans, in moist forests, and in dry, and in Ceylon, Burma, the Andamans and Nicobars" In the Punjab we have found it between the altitudes of 3,500 to 8,000 ft. above sea-level in Kulu, Bandrol, Raison, Katrain, Naggar and Manali in the Kulu Valley, Kotgrah and Simla in the Simla Hills

Food-Plants

Stebbing (1914) found it on the following plants . Sal (*Shorea robusta*), *Terminalia tomentosa*, *Hardwickia binata*, *Chloroxylon swietenia*, *Tamarix articulata*, *Acacia arabica*, Guava (*Psidium guava*), Mango (*Mangifera indica*) Beeson (1941) mentions the following as its food-plants · *Aegle marmelos*, *Alnus nitida*, *Anogeissus latifolia*, *Bauhinia acuminata*, *Bauhinia retusa*,

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Bauhinia variegata, Bombax malabaricum, Bridelia retusa, Butea frondosa, Careya arborea, Cedrela toona, Cynometra ramiflora, Duabanga sonneratioides, Eucalyptus robusta, Excæcaria agallocha, Ficus bengalensis, Grewia oppositifolia, Kydia calycina, Largerstræmia parviflora, Lannea grandis, Mallotus philippinensis, Miliusa velutina, Morus alba, Myristica andamanica, Ougeinia dalbergioides, Pentacme suavis, Pinus longifolia, Prunus communis, Pterocarpus marsupium, Pyrus communis, Quercus incana, Sapium sebiferum, Shorea assamica, Soymida febrifuga, Tectona grandis, Terminalia balerica, Terminalia myrtocarpa We collected it from Kosh (*Alnus nitida*), Cherry (*Prunus avium*), Apple (*Pyrus malus*), Crab apple (*P baccata*), Apricots (*Prunus armeniaca*), Walnut (*Juglans regia*) Plum (*Prunus domestica*), Peach (*Prunus persica*) and Mulberry (*Morus alba*)*

Description of Various Stages

Egg—Egg 2 25 mm long, 1 0 mm broad, elliptical, tapering towards either extremity, micropylar end slightly broader with a small petiole

Larva—Larva yellow with dark brown head When full-grown it measures 75 mm in length and 13 5 mm in breadth Its body is clothed in fine bristles which are abundant on the thorax and the last abdominal segment Antenna 4-segmented, 1st segment thick, rest minute with sensoria Prothorax larger than meso-thorax Thoracic legs persent. Abdominal segments possess series of tubercles on their dorsal and ventral aspects Spiracles, pit-like, elliptical, brownish

Pupa—Pupa yellow, 42 mm long, 35 mm broad, Head small, slightly deflected Thoracic sterna and 2nd and 7th abdominal segments covered with bristles Last abdominal segment bifurcated, curved dorsally

Female—Female measures 32 mm. in length, 10 mm in breadth. It is dark brown in colour with silvery or golden reflections on the elytra Antenna 11-segmented, basal segment small, rounded, 2nd segment with wrinkled surface, 3rd to 10th segments slender but thickened at their distal ends, 11th segment very thin and tapering Prothorax wrinkled and furrowed. Elytra with well-developed shoulders inner edge of each of which terminates in a small sharp spine

Male—Male resembles the female but is smaller with smaller antennæ, the last segment of each of which is flattened dorsally

Life-History

Females lay eggs from May to October They select injured areas on the bark, more often previously attacked parts of the stem, for egg-laying.

They make minute incisions on the injured edges of the bark into which they push their eggs singly and longitudinally with their ovipositors 1–5 eggs are laid daily In confinement a female laid a maximum of 92 eggs in its life-time

Eggs hatch out in 7–12 days. The young larva bites irregular holes in the egg-shell most of which it usually eats Larva on hatching feeds upon the inner layers of the bark in shallow and zig-zag galleries, but when it is a few days old it starts feeding on the inner layers of the bark and outer layers of the sapwood making shallow, wide, zig-zag and long galleries When almost grown up the larva enters the main wood through a self-prepared kidney-shaped entrance hole which later on serves as an emergence hole for the beetle. The larval stage occupies from 2 years 3 months and 5 days to 2 years and 8 months If the larva is full-grown by October it pupates after a rest of 3 to 25 days only, but when it becomes full-grown in November, the pre-pupal period may be prolonged to 4½–5 months Such larvæ pupate in the middle of April

The grub constructs a pupal chamber the distal end of which it plugs with a brownish white matter The pupa lies naked in this chamber The pupal stage occupies from 1 month 10 days to 3 months 10 days

If pupation occurs in October the beetle is formed within the chamber in December to February This beetle remains quiescent in the stem throughout the winter and spring, its resting period varying from 3 months 11 days to 5 months 2 days But when the larva enters the resting stage in winter and pupates in April, the beetle has a shorter resting period of 1 month 10 days to 1 month 17 days

TABLE I

Eggs laid	Eggs hatched	Adults emerged	DURATION OF STAGES					Life-cycle completed	
			Egg days	Larva		Pupa			
				m	d	m	d	m	d
15– 6–38	22– 6–38	26-4-41	7	28	6	2	17	34	13
17– 6–38	24– 6–38	26-4-41	8	29	3	2	17	34	9
3– 6–38	12– 7–38	26-4-41	9	27	21	3	10	33	23
13– 6–38	22– 6–38	25-5-41	9	31	23	1	10	33	12
15– 6–38	24– 6–38	25-5-41	9	30	21	1	10	32	10
4–10–38	14-10-38	30-5-41	12	29	29	1	15	29	26 –
14–10–38	26-10-38	2-6-41	12	29	19	1	17	31	18

m = months, d = days

From the date of oviposition to the date of emergence of the beetle its life-cycle is completed in 2 years 7½ months to about 3 years

Seasonal History

Beetles begin to appear on the wing towards the end of April and continue to do so upto the middle of July   They live upto the end of October   The annual calendar of activities of the pest is as follows :—

Months	Stages present
April	One-year old grubs and 2-year old resting grubs present, beetles start emerging
May-June July	Beetles keep on emerging and laying eggs Beetles continue to emerge, and oviposit-grubs present Pupæ scarce
August-September	Beetles, eggs and grubs present
October	Beetles, eggs and grubs present, old grubs pupate
November	Grubs present, old grubs start resting, few pupate
December-March	Active and resting grubs and immature beetles present

Damage

The attacked tree is recognised by the fibrous and fæcal matter which falls from the larval tunnel to the ground below   The bark around the attacked area splits up   Attacked sherry trees suffer from gummoses also.

Grubs only do damage   When young they feed upon the inner parts of the bark and the outer parts of the main wood, each grub eating out as much as half a square foot of it   Older grubs bore into the main wood and injure the inside of the stem   The damage done to the bark is more serious and when more than one grub feed in the stem the tree dies, young apple plants, however, are killed by a single grub

The damage done by the beetle itself is negligible   It feeds upon the damaged area of the stem and interior of the tunnels made by the grubs. The beetle spends the day hiding in the tunnel or beneath the injured bark , it flies at night   It has never been seen feeding upon leaves

Control

I   Prevention of oviposition

1   *Painting solignum on injured areas on the stem*—The females lay eggs in the edges of the injured bark   Therefore, when such areas were painted with solignum, the beetles refrained from laying eggs   Young apple or cherry trees should be painted every summer because young trees when attacked succumb within a month or two   Such trees are usually attacked on the stem quite adjacent to the soil   This part, therefore, must receive an occasional examination and treatment

Khan A Rahman
and Abdul Wahid Khan

Proc. Ind. Acad. Sci., b, vol. XV, Pl VIII

Fig 1 Adult a female b male

Fig 2 Egg

Fig 7 Egg slit

Fig 3 Larva a dorsal view b ventral view

Fig 4 Pupa

Fig 5 Pupal chamber
a chamber from which adult
has emerged, b chamber
from which beetle has not

Fig 8 Hole (h) through
which faecal matter (f)
is pushed out
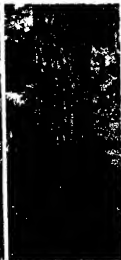
Fig 6 Emergence holes

II   *Killing grubs in their tunnels*

The tunnel is cleaned to some extent with an iron wire or knife and cotton wool soaked in kerosene oil is introduced into it   This is finally plastered with clay mud.   The fumes of kerosene oil penetrate into the tunnel, reach the grub or pupa and kill it   Others have suggested potassium cyanide crystal or a mixture of petrol and chloroform and though these chemicals are also successful they are expensive and not so readily available as kerosene oil which is also the cheapest   During 18th June–28th September 1940, 109 trees were treated with kerosene oil and cent per cent mortality of the pest was obtained.

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PHRAGMOTELIUM MYSORENSIS, A NEW RUST ON INDIAN RASPBERRY

BY M J THIRUMALACHAR

(Department of Botany, Central College, Bangalore)

THE genus *Phragmidium* founded by Link in 1824 contains several heterogenous elements and four species that were originally placed in it, have been made the types of four new genera *Hamaspora* Kornicke, *Phragmotelium* Sydow, *Frommea* Arthur and *Earlea* Arthur Of these the first three genera were segregated from *Phragmidium* because of easily distinguishable morphological characters and they are at present accepted by urediniologists The genus *Earlea* erected by Arthur (1906) to provide for those species of *Phragmidium* which lacked uredia in their life-cycle, is not accepted by Sydow (1915) or Dietel (1928), and Arthur himself now states that emphasis which was formerly placed upon the succession of spore-forms, should be transferred, because of a better understanding of the development of the rusts, to the vegetative states The presence or absence of uredia which according to Arthur, belong to the same state as telia cannot therefore be used to set off *Earlea* as a separate genus

Of the first three, the genus *Phragmotelium* is characterised by teliospores which have smooth walls and which germinate soon after ripening without any rest period Their pedicels are not well developed and they do not swell in water Aecia are further more lacking and their place is taken by aparaphysate primary uredia which form the first spore-form. As against this, the teliospores in *Phragmidium* are warty or verrucose, and they germinate only after a long rest period Their pedicels are better developed and they swell when placed in water The aecium which is the first spore-form in this genus is of the caeoma type

Sydow (1921) transferred seven species of *Phragmidium* to *Phragmotelium* and three more species are mentioned by Hiratsuka (1935) whose logical position is in the genus *Phragmotelium* He does not recognise the genus *Phragmotelium*, which according to him is a section of *Phragmidium* a view with which the present author does not agree The three species involved are *Phragmidium formosanum* Hirats *P. Rubi-fraxinifolii* Syd. (tentatively placed by Sydow in this genus as he had not seen the teliospores which

were later discovered by Hiratsuka), and *P Okianum* Hara , and it is clear that they should be transferred to Sydow's genus as *Phragmotelium formosanum* (Hirata) comb nov. *Phragmotelium Okianum* (Hara.) comb nov and *Phragmotelium Rubi-fraxinifolii* (Syd) comb. nov.

Yet another species which is new has been recently found by the author on *Rubus lasiocarpus* Smith It distinguishes itself from *Phragmotelium burmanicum* (Syd.) Syd , recorded on the same host, by its much larger and many septate teliospores. Indeed comparison of descriptions shows that this new species is easily the largest teliospored form for the genus. It

FIG. 1

Leaf of *Rubus lasiocarpus* showing infection × nat. size

resembles *Phragmotelium griseum* (Diet) Syd But this species has telio-spores with three to four germ pores, a thicker epispore, fewer septa, slightly smaller size, and above all there are no paraphyses associated with its telia, which are characteristic of the new species The name *Phragmotelium mysorensis* Thirumalachar and Mundkur is proposed for the new species

The fungus attacks only leaves (Fig. 1) causing severe blotches The life-cycle of the rust includes all the spore-forms, *viz*., pycnia, aecia, uredia and telia. Uredia and telia are formed during the months of August and September and pycnia and aecia in the months of October and November.

Pycnia

Pycnia are sub-cuticular (Fig 2) and manifest themselves macroscopi-cally as yellow specks They are mostly formed on the midrib and the secon-dary veins (Fig 5) The infected areas show slight swellings The pycnial mycelia and pycnosporophores are uni-nucleate

Aecia

Aecia are of the *cæoma* type, covered with cylindric paraphyses, which are slightly recurved (Fig 10) The aecial initials are formed in the hypo-dermal position by the concentration of hyphæ in the intercellular spaces (Fig. 9) The topmost cells of the hyphal knot which are uni-nucleate elongate and form a palisade layer of cells (Fig 3) Each of these cells cuts off one or two sterile cells which degenerate after a short period Plasmogamy takes place between any two fertile cells, the connection being established by the dissolution of the septa (Fig. 4). In many cases the fusion of superposed cells takes place (Fig 6) In any case the fusion cell elongates (Fig. 7), and by repeated cell divisions develops chains of aeciospore-mother-cells, which in turn form the intercalary cells and the aeciospores Aeciospores are spherical and minutely verrucose, with three germ pores which become dis-tinct during germination (Fig 8) The spores measure $16 \times 10\ 6\,\mu$.

Uredia

Uredia are formed associated with telia They are yellow and are surrounded by cylindric incurved paraphyses (Fig. 17) Urediospores are also yellow, echinulate (Fig 14), with a single indistinct germ pore. The spores are stipitate, binucleate and measure $16 \times 11\mu$ When germinated on slides by the method suggested by the writer (1940) the germ tube with two nuclei can be observed (Fig 15) Formation of appresorium at the tip of the germ tube is also of common occurrence.
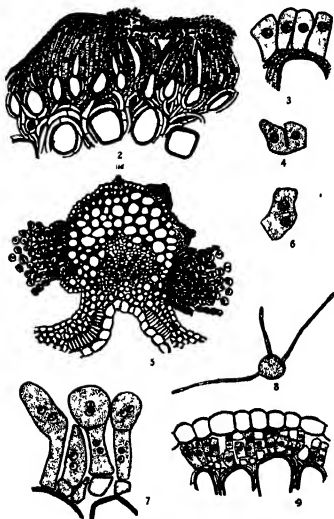
Figs 2–9

Fig. 2. Camera lucida drawing of a pycnium ×1800 Fig 3 Fertile cells of the aecium.
×1800 Figs 4 and 6 Fusion of fertile cells ×1800 Fig 5 Showing the development of
pycnia and aecia on the midrib of the leaf ×400 Fig 7 Development of aeciospore-mother-
cells. ×1800 Fig 8. Aeciospore germination showing three germ tubes. ×560 Fig. 9.
Section through an aecial initial. ×900
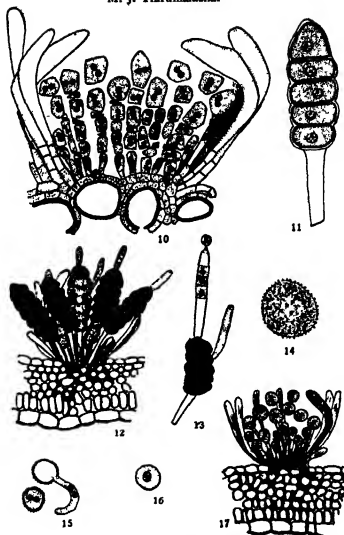
FIGS. 10–17

Fig 10   Æcium showing chains of spores and cylindric paraphyses. ×900.   Fig. 11.
Mature teliospore ×900.   Fig. 12   Section through a mature telium ×400   Fig. 13   Germinating teliospore ×400.   Fig. 14   Urediospore showing echinulate exospore. ×1260.
Fig. 15.   Showing urediospore germination. ×560.   Fig. 16.   Uni-nucleate sporidium. ×900.
Fig. 17.   Section through a uredium showing stipitate spores and paraphyses. ×400.

Telia

Telia are hypophyllous, chestnut-brown in colour and are minutely distributed on the leaf surface. They are associated with paraphyses (Fig 12). The teliospores are stipitate, and mature spores are five to six-septate. Telial initials are hypodermal from which cylindrical hyphae with two conspicuous nuclei are differentiated. Each of these divides into a stalk cell and a spore initial. The latter by a series of conjugate mitosis and periclinal wall formation forms five to six-septate teliospores. Seven septate teliospores are also developed in rare cases.

Mature teliospores are yellowish-brown, stipitate, smooth-walled with two germ pores in each cell. The cells of the teliospores are superposed, and are slightly constricted along the septa. Teliospores germinate soon after they are mature (Fig 13). In sections through the telia with spores of different maturity promycelia with basidiospores were observed in all the mature spores. It is evident that the teliospores are not resting spores. Following germination and formation basidiospores the promycelium breaks down, and the empty spore coat shrivels up and turns black in colour. Material collected for spore germination studies from old infected leaves had spores without cell contents.

Teliospore germination indicated that the promycelium emerges through the germ pore and at one time more than one cell of the teliospore germinates simultaneously. The yellowish-brown contents of the teliospores migrate into the promycelium. A four-celled promycelium is formed giving rise to basidiospores. The basidiospores are thin-walled, spherical and uni-nucleate (Fig 16). They measure $11 \times 10~5\mu$.

Leaves of *Rubus lasiocarpus* with freshly erupted telia were taken and suspended over young plants raised from cutting under rust-free conditions. The leaves of host plant were kept moist by spraying them with water. The plants were enclosed under bell-jars. After twenty days small discolourised spots were observed indicating the first sign of infection. Eight days after the pycnia were observed appearing macroscopically as orange yellow specks. Further development could not be followed owing to the death of the host plant.

Description of the Rust

Phragmotelium mysorensis Thirumalachar and Mundkur, Spec nov

Pycnia epiphyllous, sub-cuticular, without paraphyses. Aecia of the *caeoma* type, covered with long cylindric paraphyses, aeciospores yellow, oval or spherical, minutely verrucose, with three germ pores, measuring $16 \times 10~6\mu$. Uredia hypophyllous, associated with telia, minute, sparsely

distributed, surrounded by incurved cylindric paraphyses, urediospores yellow, spherical, echinulate measuring $16 \times 11\mu$, with an indistinct germ pore. Telia erumpent, chest-nut brown, sparsely distributed on the leaf surface, provided with paraphyses, teliospores stipitate, five to seven septate 5-celled spore measuring 64·5 to 73·5 × 23·2 μ, 6-celled spore 77·4-85 × 23·2 μ, and 7-celled spore 82-88 × 23·4 μ, yellowish-brown smooth thin-walled, with two indistinct germ pores in each cell, pedicel hyaline, not swelling in water measuring 40 to 48μ, spores germinating *in situ* immediately Basidiospores thin-walled, spherical, measuring $11 \times 10 \cdot 5\mu$.

Hab on leaves of *Rubus lasiocarpus* Smith leg, Thirumalachar, Nandi Hills, Mysore State, 16–2–1941   Type deposited in Herb *Crypt Ind Orient* of the Imperial Agricultural Research Institute, New Delhi *Phragmotelium mysorensis* Thirumalachar et Mundkur Spec nov

Pycnia epiphylla, sub-cuticularia, aparaphysata, Aecia cæmoidea, paraphysatibus, longis, cylindricis tecta, aeciosporæ flavidæ, ovatæ vel sphericæ, tenuiter echinulate, tribus germinationis sporis instructæ, magnitudinis 16×10 6μ Uredia hypophylla, mixta cum telus, minuta, sparse-disposita, cincta paraphysibus curvatis, cylindricis, urediosporæ flavidæ, sphericæ, echinulate, magnitudinis $16 \times 11\mu$, præditæ uno germinationis poro indistincto   Telia erumpentia castaneo-brunnea, sparse distributa per foliorum faciem, paraphysata; teliosporæ pedicellatæ, 5–7 septatæ; sporæ 5-cellulatæ magnitudinis 64·5–73·5 × 23·2μ; Sporæ 6-cellulatæ, magnitudinis 77·4-85·0 × 23·2μ *et* sporæ 7-cellulatæ, 82–88 × 23·4μ, sporæ omnes flavidobrunneæ, tersis *et* tenuibus parietibus ornatæ, et singulæ sporæ duobus germinationis pores indistinctis instructæ, pedicellus hyalinus est neque tumescit in aqua, magnitudinis 40–48μ Sporæ statim germinantes, basidiosporæ tenuibus parietibus ornatæ, sphericæ, magnitudinis $11 \times 10 \cdot 5\mu$

Hab in folus *Rubi lasiocarpi* smith, leg. Thirumalachar, Nandi Hills, Mysore State, India, 16–2–1941

Conclusions

The absence of the cæoma type of aecia was one of the chief reasons that led Sydow (1921) to establish the genus *Phragmotelium*, a primary aparaphysate uredium taking its place as the first spore-form. But *Phragmotelium mysorensis*, it will be noted, is characterised by the presence of an aecium of the cæoma type and by the absence of primary uredia. The teliospores with smooth spore walls and with a capacity to germinate immediately; and with pedicels that cannot swell in water, leave however no doubt as to the position of this rust in that genus

That there would be transition forms between *Phragmidium* and *Phragmotelium* cannot be doubted, and there is nothing surprising in coming across species with overlapping characters. For instance species of *Phragmidium* formerly placed in the genus *Earlea* have teliospores with smooth walls, though they germinate after a long rest period. Paralleling this we have *Phragmotelium mysorensis* in which the primary aecia are replaced by an aecium, though the other characteristics distinguishing the genus *Phragmotelium* are all present in the species

The author desires to express his gratitude to Dr B B Mundkur, Imperial Agricultural Research Institute, New Delhi, for help in writing this paper, in identifying the fungus, and in obtaining the latin diagnosis, and to Dr M A. Sampathkumaran, Professor of Botany, University of Mysore, for encouragement and guidance

Summary

1   *Phragmotelium mysorensis* is a new species of rust attacking the leaves of *Rubus lasiocarpus* Smith

2   All the four spore-forms, viz, O, I, II and III occur on the same host

3   Pycnia are sub-cuticular, and aecia are of the cæoma type with paraphyses. Development of cæoma and the initiation of the dicaryon phase has been studied

4   Uredia are hypophyllous, pulverulent, covered with incurved paraphyses

5   Telia are hypophyllous, associated with the uredia and covered with paraphyses   Teliospores are five to six septate   Teliospores are smooth, thin-walled, with two indistinct germ pores in each cell.   Pedicels are hyaline and do not swell in water   Teliospores germinate soon after maturity, and form sporidia which are uni-nucleate

6   Sporidial infections indicate that the rust is autoecious

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FEMALE GAMETOPHYTE AND EMBRYOGENY IN *CYMBIDIUM BICOLOR* LINDL.

By B G L SWAMY

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(Communicated by Rao Bahadur G N Rangaswami Ayyangar)

Introduction

AN account of microsporogenesis and development of the male gamete in *Cymbidium bicolor* was recently published by the author (1941) The present work deals with the megasporogenesis and embryogeny

The material was fixed in formalin-acetic-alcohol and embedded in paraffin after the usual methods of dehydration and infiltration Sections were cut between 8 and 20 microns and stained in Heidenhain's iron-alum hematoxylin followed by a counterstain of either eosin or light green in clove oil.

Megasporogenesis

Sections through even the fully opened flowers rarely showed the nucellar protuberances, in which the differentiation of the archesporium and its further development is initiated only after pollination The sign of successful pollination is the enlargement of the gynostegium and material was collected at various stages for microscopic examination

The archesporial cell is hypodermal in origin and is easily differentiated by its large size, conspicuous nucleus and rich contents Further development is seen only in material collected ten days after pollination Even at the archesporial cell stage pollen tubes can be observed along the placental ridges.

The archesporial cell functions directly as the megaspore mother cell (Fig. 1) The nucleus passes through the usual stages of the meiotic division (Fig. 2), and a dyad is formed (Fig. 3). The chalazal cell enlarges (Fig 4) and gives rise to an 8-nucleate embryo-sac (Figs 5–9) The development of the embryo-sac thus conforms to the *Allium*-type.

In some cases the nuclear divisions are suppressed at the antipodal end or they show a belated development, so that occasionally only 6-nucleate

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* The name of *Cymbidium bicolor* has been revised in Gamble's Flora as *Cymbidium aloifolium* (Gamble Fl Mad Pres , Part VIII, p. 1436)
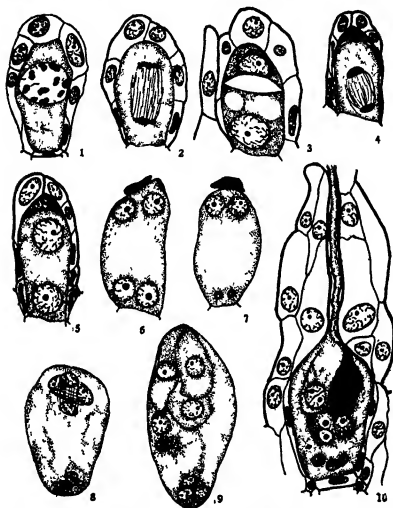
FIGS 1–10

Fig. 1 Megaspore mother cell in diakinesis Fig 2 Megaspore mother cell in anaphase of first reduction division. Fig. 3 Dyad Fig 4 Lower dyad cell dividing and the upper degenerating. Fig 5 Two-nucleate embryo-sac Fig 6 Four-nucleate embryo-sac Fig. 7 Four-nucleate embryo-sac showing the difference in size between the chalazal and micropylar nuclei Fig 8. Eight-nucleate embryo-sac. Fig. 9 Mature embryo-sac Fig 10 Fertilization. Magnification of Fig. 1, ×1260, of the rest × 560.

embryo-sacs are seen Usually the antipodal nuclei are smaller in size than the micropylar ones This difference is often noticeable even at the 4-nucleate stage (Figs 7–9)

Fertilization and Endosperm Nucleus

The germination of the pollinium and the formation of the tube nucleus and the male nuclei has already been described by the writer in a previous paper (Swamy, *loc cit*) Fertilization is porogamous (Fig 10) The male nuclei which are bean-shaped during their passage through the pollen tube become spherical after being discharged into the embryo-sac

One of the male nuclei fuses with the egg nucleus and forms the zygote The other enters into triple fusion with the partially fused polar nuclei (Fig 10) Even before they complete fusion the three nuclei show slight hypertrophy and sometimes become five to six times larger than before (Figs 11, 12, 29 and 30) But gradual degeneration sets in, though the remains of the nuclei persist even up to late stages of embryogeny

Embryo

The fertilized egg divides after a long rest, sometimes extending up to nearly two months after fertilization The first wall is transverse (Fig 12) and very often oblique (Fig 28) and even vertical (Fig 29) The next three to six divisions are very irregular, the walls being laid down without any definite order (Figs 13, 14 and 15) One of the cells at the chalazal end of this resultant irregular mass continues to divide further and forms a linear row of 6–10 cells (Figs 16, 17 and 18) The chalazal two or three cells of this proembryo undergo vertical divisions (Figs 19 and 20) followed by more anticlinal and periclinal divisions (Figs 21 and 22) and form the embryo (Figs 23, 24, 25 and 26)

Some of the suspensor cells at the micropylar end begin to elongate and present a fluffy appearance (Figs 16, 17, 18, 22, 23, 24, 25, 26 and 35) Some of them grow into the micropyle, and some others surround the developing embryo They do not extend in any case out of the outer integument but occupy the space available in it (Fig. 25 *a*) These cannot be considered strictly haustorial as they do not reach any nutritive region even during the late stages of embryogeny nor do they digest the surrounding tissue during their elongation This type of elongation is restricted to only 5 to 7 cells at the top In a mature seed at the time of opening of the fruit, these elongated cells are degenerated and only their dwindled remains are seen.
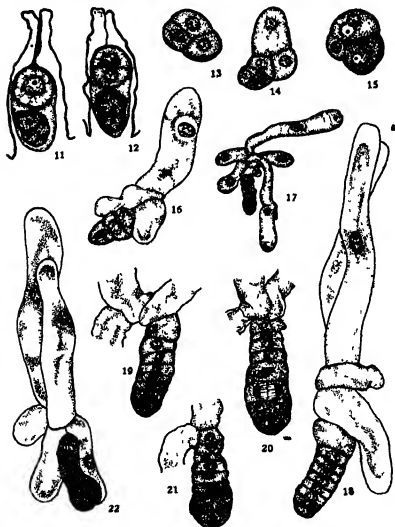
FIGS. 11–22

Fig. 11 Zygote and the hypertrophied endosperm nucleus. ×560 Fig. 12. Two-celled embryo. ×560 Figs. 13, 14 and 15 Early stages of embryo development. ×560 Figs 16 and 17. Later stages, note the enlargement of the suspensor cells towards the micropylar end. ×400. Figs. 18, 19, 20, 21 and 22. Stages depicting the subsequent development of the embryo. Fig. 18, ×560. Fig. 19, ×400. Fig. 20, ×300. Fig. 21, ×160 Fig. 22, ×160.
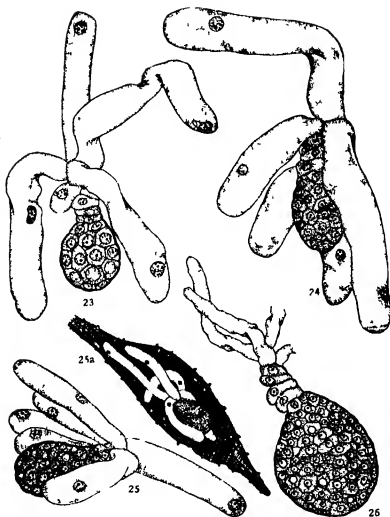
Fig 23 A proembryo with four elongated cells towards the micropyle   200   Figs 24 and
25 Later stages of the same with five and six elongated cell towards the micropylar end
200 Fig 25a An optical section passing through the longitudinal plane of the ovule
showing the position of the embryo and prolongations of the suspensor cells   160 Fig 26
Mature embryo at the time of dehiscence of fruit   200

Figs 27-36

Polyembryony

Occasionally two embryos are seen in a single ovule Their development has been traced in detail and is shown in Figs 27 to 36 In some cases where the first division of the zygote is vertical (Fig 27) or oblique (Fig 28), the two resulting cells get separated (Figs 27, 29) and each develops independently into an embryo (Figs 30, 31, 32, 33, 34, 35 and 36)

Usually one of the two embryos is slightly smaller than the other (Figs 35 and 36), probably due to want of space or nutrition and its tardy development as compared with its fellow Out of 2 000 mature seeds examined under the microscope 27 contained double embryos, i e , about 2%

The mature seed (Fig 36) has a single layer of the integument composed of translucent cells with deep brown reticulations The seed measures about $13 \times 52 \mu$

Conclusions

The *Allium*-type of development in terrestrial orchids was first reported by Vermoesen (1911) and subsequently by others Stenar (1937) has recently shown the same type of development in *Acroanthes monophyllus* Among strictly epiphytic orchids this type of development has now been recorded in *Cymbidium bicolor*

The vertical division of the zygote is rare among angiosperms and has been recorded only in a few plants belonging to the *Amentiferæ* It has been reported in *Leitneria floribunda* (Pfeiffer, 1912), in *Sassafras* (Coy, 1928) and in a few members of Loranthaceæ (Schnarf, 1931) Vertical divisions of the embryo upto 6-celled and 8-celled stages have been recorded in *Balanophora dioica* (Ekambaram and Panje, 1935) and in *B abbreviata* and *B indica* (Zweifel, 1939) As mentioned before, in *Cymbidium bicolor* the vertical division may lead to the origin of two embryos and results in cleavage polyembryony

Summary

1 The hypodermal archesporial cell directly functions as the megaspore mother cell

2 The mode of development of the embryo-sac conforms to the *Allium* type Sometimes only 6-nucleate embryo-sacs are seen due to a reduction of the number of divisions at the chalazal end

3 The pollen tube enters through the micropyle Double fertilization has been observed

4   A long suspensor is formed during the development of the embryo
The terminal cell forms a filamentous proembryo which gives rise to the
embryo

5   Some of the suspensor cells at the micropylar end elongate and
enlarge to enormous proportions   They are however not haustorial and
degenerate in later stages

6   Cleavage polyembryony is reported and its mode of origin and deve-
lopment traced   The plural embryos are monozygotic

The author takes this opportunity to express his sincere gratitude to
Dr P Maheshwari, D Sc , for his valuable help

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A STUDY OF THE LIFE-HISTORY AND CONTROL OF *BATOCERA HORSFIELDI* HOPE (LAMIIDÆ COLEOPTERA)—A BORER PEST OF WALNUT TREE IN THE PUNJAB

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Introduction

A LONG-HORNED beetle *Batocera horsfieldi* Hope, is a serious pest of walnut in the Kulu Valley and Simla Hills in the Punjab and of " walnut and alder in the Darjeeling Himalayas and of oak in the Kumaon Himalayas " (Beeson, 1941) In addition to these plants Beeson (1941) also found it on *Salix tetrasperma*, *Trema amboinensis* and mentions its occurrence on *Parlowina tomentosa* in Japan In the Kulu Valley and Simla Hills, though present between the altitudes of 3,500 ft to 8,000 ft above sea level, we have found it to be more destructive between the altitudes of 5,000 to 8,000 ft

Description of Stages

Adult (Fig 1 *a*, *b*) –Beeson's (1941) description of the adult is below

" Beetle black with fine ashy or yellowish grey pubescence, pronotum with 2 elongate white or yellowish spots, elytra with numerous shining black tubercles of the base, and several rounded or broken elongate white marks extending to the truncate apex, scutellum white or yellowish " Males and females can be separated as follows --

Female (Fig 1 *a*)	Male (Fig 1 *b*)
(1) 60 mm long and 21 mm broad	53 mm long and 17 5 mm broad
(2) Antennæ 70–76 mm long, as long as body	Antennæ 76–86 mm long longer than body
(3) Anal extremity narrower and without any hairs	Anal extremity blunt and clothed in brown ash hairs

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Egg (Fig 2)—Egg is oval in shape and brownish in colour with a thick and leathery chorion , it measures 11 mm in length and 4 5 mm in breadth

Larva (Fig 3 a,b)—The larva is pale yellow in colour and when full-grown it measures 90 mm in length The head is small in size, triangular in shape and dark brown in colour Antennæ are minute, two segmented, and are embedded in oval pits Prothorax is the broadest segment, measuring 19 mm in width, from here the body tapers gradually to the anal segment, which is the narrowest measuring 13 mm in breadth Prosternum is broad, dark yellow and studded with numerous tubercles Thoracic legs absent The newly hatched larva resembles the full-grown one but is smaller, its length being 10 5 mm and its greatest width (across the prothorax) 4 0 mm

Pupa (Fig 4)—The pupa is creamy white to pale yellow in colour and is 55 mm in length and 20 mm in breadth It lies in a specially prepared pupal chamber (Fig 5)

Life-History and Habits

Under field conditions this pest completes its life-cycle in 22-32 months Emergence of the adults from the pupal chambers commences in the early parts of June and continues till about the end of July, at the end of October or early November these adults die after copulation and oviposition the adults emerge by cutting out a 4-5″ long, curved passage from the chamber through the stem which terminates (on the stem) in a circular hole of 75″ to 1 0″ diameter (Figs 5 a, 6) The adults rest on their food-plant and when disturbed they stridulate They feed on the bark of young twigs, but the damage done by them is negligible

A female lays 55 to 60 eggs singly in an upright position in specially made transverse slits (Fig 7) in the bark of the trunk or main limbs of the food-plant Eggs hatch within a period of 8 15 days, depending upon the season As soon as the head of the larva comes out of the egg-shell, it begins to bore into the tree, the larva never leads an exposed life Frass issuing from the egg-slit always indicates the hatching of the egg The young larva feeds on the inner side of the bark in shallow, narrow and zig-zag tunnels After a few days it feeds both upon the inner side of the bark and outer regions of the sap wood making shallow, wide, and zig-zag galleries It makes irregular tunnels and throws out quantities of chewed up plant tissue mixed with fæcal matter (Fig 8 f) through holes (Fig 8 h) In heavily infested trees heaps of chewed up fibre mixed with fæcal matter are found beneath the hole, large quantities of frass also remain in the tunnels, and under the bark

The larval stage lasts for 20-25 months The full-grown larvæ do not show any activity during winter months, i e , from October-March The pest has a distinct prepupal stage which lasts 50-182 days depending upon the season Prior to pupation the larva constructs a lunar-shaped chamber in the main stem (Fig 5 b) In this chamber it spends its prepupal and pupal stages The pupal stage occupies 46 to 90 days The beetle does not leave the pupal chamber at once on emergence, but rests in it for a varying period of 5-6 months It ultimately emerges out through a 4-5″ long curved tunnel which terminates, as pointed out above, in a circular hole of 75-1 0″ diameter The plant heals up the tunnel and the hole in 2-3 years

Grubs less than one year old continue feeding throughout winter, they hibernate as full-grown larvæ in the pupal chamber

Table below gives the complete life-cycle of the pest —

m = months, d = days

Eggs laid	Eggs hatched	Adults emerged	Duration of stages					Life-cycle completed	
			Egg days	Larva		Pupa			
				m	d	m	d	m	d
18- 7-36	26-7-36	1-6-38	8	20	19	1	16	22	13
10- 9-37	24-9-37	5-6-40	14	24	24	3	0	32	25
3- 8-37	18-9-37	17-7-40	15	23	24	2	0	32	13
13- 6-39	21-6-39	3-6-41	8	21	24	1	18	23	21
13- 6-39	21-6-39	5-6-41	8	21	24	1	20	23	23
13- 6-39	21-6-39	7-6-41	8	21	24	1	22	23	25

Methods of Control

The control measures against this pest are directed against the beetles, eggs and the larvæ and consist in (1) Catching and destroying the adults, (2) destroying eggs and young larvæ in egg-slits, (3) treating of holes in the stem and main branches with potassium cyanide or kerosene oil

Catching and destroying the adults —The adult beetle (Fig 1 a, b) is a large-sized, stoutly built insect It rests on tree trunks during June-October when it should be searched out, captured in a handnet and destroyed

Destroying eggs and young larvæ in egg-slits —They are laid on the main stem usually upto 15 ft. from the ground, they may also be laid in thick branches The position of the egg-slit is revealed when the young larvæ bore into the stem and push out frass The eggs and grub should be destroyed by probing the egg-slit with a knife or an iron wire

Treating of holes in the stem and main branches with potassium cyanide or kerosene oil—This method is best suited for the control of the older grubs. Before injecting either of the poisons, frass should first be removed from the entrance of the hole and a pointed wire should be pushed into the hole to clear the tunnel. Two grams of potassium cyanide or a plug of cotton wool soaked in kerosene oil should be inserted into the hole and the hole closed with mud. After each treatment heaps of frass should be removed from under the treated plant.

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INDIAN WATER MOULDS—III

By Abdul Hamid, M Sc

(From the Botany Department, Panjab University, Lahore)

Introduction

Water moulds are the saprophytic inhabitants of water which is calm or slowly flowing They flourish well in sewer waters where organic matter is plenty and there is less of external disturbances

The author collected some interesting forms from the tanks adjoining the *Mandirs* and *Gurdwaras*, here, when it was breezy, the infected insects would come to rest near the edge of the tank, thus making collection easy

Small ditches near the " Rajbahas " (a small canal distributary) where green organic matter is abundant are the favourite resorts of the forms like *Pythiogeton* and others They are mostly found growing on submerged plants, twigs and fruits in springs, creeks, troughs, ditches and moats puddles and pools

Months of October and November are doubtlessly favourable for their growth but the reproductive phase extends upto the middle of December and after that the severity of cold and frost brings them to their resting stage The other favourable period ranges from the middle of February to May when the rising spring temperature spurs them into activity again During rains, they get disturbed and it becomes difficult to collect specimens, but rains are helpful in the distribution and migration of the species

Two papers on Indian Water Moulds have been published by Chaudhuri and Kochhar (1935)[1] and Chaudhuri and Lotus (1936)[2] In the present paper, the author has described only those forms that have not been previously reported in India

[1] " Indian Water Moulds—I," *Proc Ind Acad Sci* , August 1935 2, No 2

[2] " Indian Water Moulds—II," *ibid* , April 1936, 3, No 4

Considerable difficulty was felt in following the spore discharge as in some forms it took place late at night and continued for 7–8 hours Similarly discharge of the eggs had sometimes to be followed during night

Culture The usual water culture methods of cultivation were adopted with slight modifications A boiled house-fly was placed for about 36 hours in the specimens of water collected from different places When a white halo appeared round the fly, it was taken out and washed in several changes of sterilized water The first infection invariably consists of a mixture of species, hence it is necessary to isolate them and grow them in pure culture Pure culture from single sporangium was made by taking out a mature sporangium which was washed in acidulated water and finally inoculating it on to the bait In a couple of days the hyphæ radiating from the bait were seen After giving two or three washings in sterilised water, the culture was transferred to another pair of sterilised petri-dishes containing sterilised water This process was repeated after every four days and culture transferred to a fresh pair of dishes

Various baits were tried but boiled maize seed proved to be the most favourite bait The maize seed is freshly autoclaved to such an extent that it bursts exposing fresh milk-white endosperm Grapes have been tried as baits but did not prove very successful The hyphæ grew out but soon died forming a gelatinous mass Egg yolk was successful and occasionally very useful in the production of oogonia Fly and other insects were equally useful Uncooked potato pieces served a useful purpose in the cultivation of the present forms This was found to be the favourite medium for the new form described in this paper

Staining The staining process is preceded by killing and fixing operations The fixing and killing was done in acetic formalin The fixative contained 5 c c of acetic acid, 10 c c of formalin and 85 c c of water

Various staining methods have been tried Eosin alone gave very faint colour, erythrosin did not give satisfactory results Consequently the use of mordants was thought advisable Various iron salts were tried but without any satisfactory results Eventually 05% solution of aluminium sulphate was prepared and the fixed material was put in it for 2–6 hours The material was taken out, washed twice or thrice in water and put in a strong solution of erythrosin, to which a few drops of glycerine had been added The material after four hours was removed and thickened in glycerine and mounted in jelly or glycerine and sealed with canada balsam This was found to be quite satisfactory Cotton blue-lactic acid stain has also been tried and gave good results
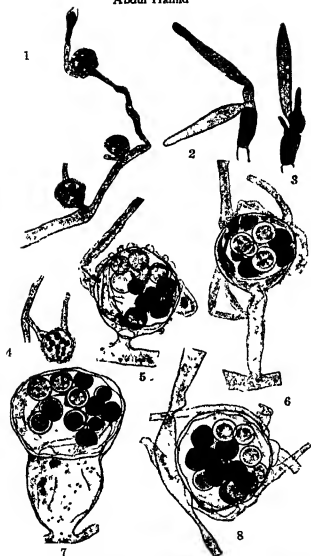
PLATE I   *Achlya oblongata*

FIGS 1–8   Fig 1   Hypha and arrangement of oogonia ×112   Figs 2–3   Zoosporangia and chlamydospores. ×105   Fig 4   Oogonium with diclinous antheridia ×112   Figs 5–6 Oogonium with diclinous antheridia ×300   Fig 7   Oogonium with bulbous base. ×300 Fig. 8   Oogonium with large number of eggs ×300

I Family *Saprolegniaceæ*

1 *Achlya* Nees v Essenbeck

A oblongata de Bary, *Bot Zeit*, 1888, **46**, 646, P 10, Figs 7 9 (Plate I)

Growth somewhat dense, reaching upto 2 cm on maize in distilled water Hyphæ branching, tapering towards the apex (Fig 1) The branches are smaller in diameter The diameter of the branches ranges from 2 77–22 2 μ, at times upto 27 7 μ, generally it is 11 1 μ Zoosporangia thicker than the vegetative hyphæ, mostly club-shaped, 88 8–136 1 μ long (Fig 2) chlamydospores few but with thick and dense contents and various shapes (Figs 2, 3) Zoospores coming out in groups stick at the tip of the sporangium

Oogonia somewhat rounded, plentiful and on short lateral stalks The stalks not thicker than oogonia and not equal in length to the diameter of the oogonia The diameter of the oogonia varies from 55 5–111 1 μ, and generally it is from 83 3–108 μ Wall smooth and thin Egg 1–16 (Figs 4–8), diameter 20–27 μ The contents of the eggs are thick and granular Antheridia always diclinous (Figs 4–8), mostly covering the whole oogonium The oogonia are borne alternately and in racemose manner

Growth in culture —On house-fly in distilled water—hyphæ abundant, club-shaped sporangia many Spores discharged through an apical pore On egg yellow in distilled water—vegetative growth luxuriant On potato in leucine (0 1% in water)—gemmæ (chlamydospores) formed, growth moderate On maize—growth vigorous, when put in leucine water (0 1%) hyphæ develop zoosporangia, oogonia not formed Growth on fly in leucine water (0 1%) was vigorous, oogonia produced after 2 weeks, but with 0 1% potassium phosphate and potassium nitrate growth was scanty

The specimen differed from the description of de Bary's specimen in the presence of certain clavate sporangia and less number of gemmæ and eggs, but these differences are not striking enough as to justify its position as a new species

Collected from Lahore in April, 1936

2 *Achlya andracomposita* sp nov (Plate II)

Growth not delicate, hyphæ 13 8–41 6 μ, branched, zoosporangia 400–750 μ long, cymosely branched (Figs 2–4) Zoospores 8 3 μ in diameter,

Figs 1–8   Fig 1 Hypha and arrangement of oogonia ×98   Fig 2 Arrangement of
zoosporangia ×90 Figs 3–4 Chlamydospores and zoosporangia ×5 Fig 5 Oogonium
showing diclinous and androgynous antheridia ×285 Fig 6 Oogonium showing diclinous
antheridia ×487 Fig. 7 Oogonium showing diclinous and androgynous compound anthe-
ridia ×285 Fig 8 Oogonium showing antheridial arrangement ×475

Oogonia globular, borne racemosely (Fig 1), diameter 61–75 μ, walls smooth Stalks almost equal in length to the diameter of the oogonia The number of eggs varies from 4–11, generally 6–8 (Figs 5–7) Diameter of eggs 20 8–30 5 μ, mostly 25 27 μ, centric, antheridia androgynous as well as diclinous (Figs 5–7), diclinous condition more abundant Antheridia compound supplying more than one oogonia Sometimes antheridia arise from the base of the stalk of oogonia

Achlya androcomposita

Hyphæ robustiores, 13 8–41 6 μ diametro ramosæ Zoosporangia 400–750 μ longa, cymose ramificata Zoosporæ 8 3 μ diametro Oogonia globosa, in racemis, diametro 61–75 μ, pariete polito Stipites longitudine diametro ovorum fere æquales Numerus ovorum variabilis, 4–11, generatim 6–8 Ova diametro 20 8–30 5 μ, plerumque 25 27 μ Antheridia androgyna aut diclina, diclina conditio frequentior Antheridia composita, pluribus oogoniis suppeditantia Interdum antheridia ab imo oogonii stipite orientia

Growth in culture —On insect in water—growth meagre but stout On potato and water—slight growth but numerous oogonia On egg yolk—growth dense Zoosporangia abundant On maize in water—slight growth On maize in leucine water (0 1%) vigorous growth and oogonia formed—after seven days Nitrate solution—retarded the growth

Achlya androcomposita has diverse affinities The presence of (a) unpitted wall of oogonia, (b) compound antheridia, (c) diclinous as well as androgynous antheridia, (d) measurements of eggs (20–30 5 μ) and oogonia 61–75 μ, (e) the number of eggs (4–11), and (f) the length of the zoosporangia, mark it to be a new species of *Achlya* It approaches *A dubia* and *A megasperma* in unpitted oogonia, but differs from these in having both diclinous and androgynous compound antheridia and larger number of eggs It has nearly the same number of eggs as *A Klebsiana* but the position of antheridia is strikingly different

Collected from Amritsar, Lahore and Hoshiarpur

II Family *Pythiaceæ*

1 *Pythiogeton* Von Minden (1916)

· *Habitat* —The fungus was isolated from samples of water containing decaying twigs, obtained from sewers and ponds, in the vicinity of Lahore

Morphological characters of the genus —Hyphæ delicate, generally non-septate, sporangia of different ages occurring in clusters Sporangia

asymmetrical, club-shaped or curved, attached to delicate hyphæ at right angles to the axis At first a tubular structure is sent out with a mucilage plug at the tip The plug gradually dissolves and the contents (plasma) are poured out forming a cloudy mass later on breaks into swarm spores Sporangia proliferate frequently Re-innovation occurring in some cases twice or thrice Each sporangium having a tube of its own Although sexual organs have been described in the three species of *Pythiogeton*, viz, *P utriforme*, *P transversum*, *P ramosum* but the sexual organs have not been observed in the specimen described here

Pythiogeton sterilis sp nov (Plate III)

Characters as above Delicate hyphæ, break on slightest disturbance, non-septate, 2 5–3 8 μ in diameter Sporangia in swarming numbers, attached to the hyphæ at right angles to the axis, 100–313 μ × 30–60 μ (Figs 1, 4–6), spore mass which is still in undifferentiated condition is poured out and during an interval of 7–8 hours (Figs 4–6) The spore mass is sent out through a tubular structure by the dissolution of the mucilage plug at the tip of the tube (Figs 7, 9–10) This discharge usually occurs at night Occasionally plasma of the sporangium is discharged into a vesicle (Fig 8) Internal proliferation frequent and a number of times (Figs 10–12) Occasionally a portion of the spore mass was delimited inside the sporangium (Figs 14–15) No sexual organs observed

Pythiogeton sterilis

Characters ut supra Hyphæ teneræ, minima conturbatione confrin-gentes, non septatæ, diametro 2 5–3 8 μ Sporangia cumulata hyphis angulo recto ab axi orientibus affixa, 100–313 × 30–60 μ Sporarum massa non-differentiatarum intervallo 7–8 horarum effunditur Ejectio sporarum massæ fit habitualiter nocte per tubulum aliquem dissolutione obturamenti mucilaginei in extremo tubulo Quandoque sporarum plasma vesiculo aliquo injicitur Interna proliferatio frequens pluribusque vicibus (in eodem sporangio) Interdum portio sporarum massæ in sporangio delimitatur Organa sexualia non visa

Growth in culture —Isolated from decaying twigs in dirty sewer and pond water with potato pieces (fresh) Later on developed favourably on maize in water Attempts to cultivate it on fly and leucine were not futile, though the growth was scanty, dwarf but abundant fruit bodies were formed It offered great resistance to its cultivation on other media like insect, egg yellow, potassium nitrate and potassium hydrogen phosphate

PLATE III *Pythiogeton sterilis*

FIGS 1–7 Fig 1 Mature sporangium showing lateral stalk ×487 Fig 2 Sporangium borne directly between hyphal continuity ×487 Fig. 3 A developmental state of sporangium ×337 Figs 4–5 Mature sporangium bursting at the tip ×187 Fig 6 Cluster of sporangia ×112. Fig. 7 An empty sporangium showing the discharge tube and a mature sporangium just before the tube formation. ×187
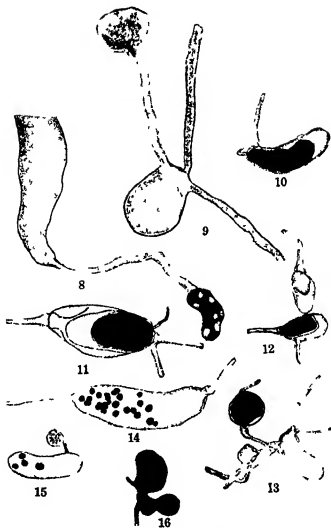
PLATE III   *Pythiogeton sterilis* (continued)

FIGS 8–16   Fig 8   Sporangium discharging its contents into a compact vesicle ×326
Fig 9   Long stout discharge tube pouring out spore mass ×487   Figs 10–12   Sporangia
showing internal proliferation ×300   Fig 13   Empty sporangium with vesicle and discharge
tube ×300   Figs. 14–15   Some of the spores delimited inside the sporangium ×300   Fig 16
Abnormal gemma like sporangium ×300

Hyphal growth without these sporangial bodies was delicate and silky Best temperature for growth was 22–25° C Leucine 0 1% invigorated the growth in culture Best period of vegetative activity was found to be January–March Although it can resist high summer temperature but the bacterial growth kills the fungus Daily baths with fresh water made the fungus rather sturdy and bacteria-free

P sterilis differs from the other three varieties, viz, *P uriforme*, *P ramosum* and *P transversum* in the absence of sexual organs for which reason it has been placed under a new species

Collected from Lahore

Summary

This is the third contribution in the series "Indian Water Moulds" Two species of *Achlya*, viz, *Achlya oblongata* and *A androcomposita* nov sp and one species of *Pythiogeton*, viz, *P sterilis* nov sp, have been described

INDIAN WATER MOULDS—IV

By H. CHAUDHURI AND M. L. BANERJEE

(From the Botany Department, Panjab University, Lahore)

Fam *Saprolegniaceæ*

Protoachlya Coker

This genus was established on a species collected at Chapel Hill (America) and previously described as *Achlya* Coker had first named it as *Achlya paradoxa*, but later changed this to *Isoachlya paradoxa* Finally in 1923 he established it as a new genus and named it *Protoachlya paradoxa*

Generic characters – Hyphæ more delicate than in *Achlya*, sporangia subcylindrical to clavate or flask-shaped, blunt and usually thickest beyond the middle, proliferating like a cyme as in *Achlya*, and also, less frequently, by growth through the empty sporangia as in *Saprolegnia* Spores diplanetic on emerging ciliate and all or some showing sluggish or less often active motion, some remaining attached in an irregular clump to the tip of the sporangium Oogonia borne singly, the great majority on short lateral stalks from the main hyphæ and with or without a few pits, eggs usually few, centric Antheridia androgynous or diclinous, typically pyriform with their tips applied to the oogonium Gemmæ spherical to pyriform or elongated Vegetative behaviour not noticeably different from the other genera

Habitat —The soil samples along with water were collected from a drain in Lahore and from Hiran Minar tank, Sheikhupura, during the month of November, 1938

P paradoxa Coker (Plate I)

Hyphæ slender, spirally twisted, little branched, largest $60\,\mu$ at the base, others smaller generally between $29\ 84$–$44\ 76\,\mu$, zoosporangia mostly club-shaped, $508\ 2$–$281\,\mu \times 62\ 68$–$38\ 5\,\mu$, rounded at the top with a distinct short papilla (Figs 1-4), secondary zoosporangia usually formed by internal proliferation through the older ones, new zoosporangia formed entirely outside the primary zoosporangia (Figs 2-4), rarely formed by cymose branching, zoospores biciliate, developed in several rows and all of the same zoosporangium behaving in the same manner, diplanetic Chlamydospores pyriform or elongated with thick walls Chlamydospores are developed mostly on the tips of hyphæ, either in chains or terminal (Figs 8, 9)

216
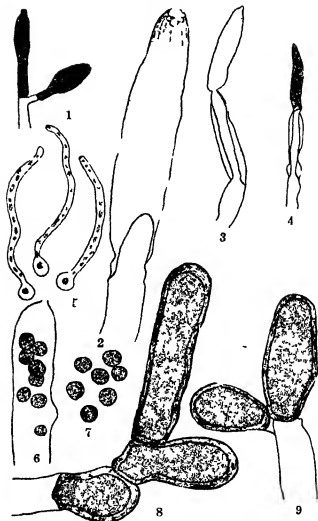
PLATE I *Protoachlya paradoxa*

FIGS 1–9 Fig 1 Cymose branching of zoosporangia ⟩105 Fig. 2 Zoosporangium showing ruptured apex ×525 Fig. 3 Secondary zoosporangium formed through the primary zoosporangium ×109 Fig 4 Secondary zoosporangium with mature zoospores ×109 Fig. 5 Germinating zoospores ×487 Fig 6 A portion of zoosporangium showing encysted zoospores. ×487. Fig 7 Encysted zoospores ×525 Fig. 8 Three chlamydospores in chain. ×487. Fig. 9. Two chlamydospores ×487.

They are mostly elongated and rarely globular   They are thick-walled which measure 3 2 μ   A few oogonia and antheridia developed

Cultural characters – In pure culture the authors failed to get any oogonial and antheridial formation, but in some contaminated dishes, a few oogonia and antheridia were formed, but there were more or less, disorganised

On egg albumen in sterilised water—growth extensive, reaching a length of 1¼ inches from the margins of the bait, plenty of zoosporangia formed ; on egg albumen in tap water—growth vigorous, zoosporangia and chlamydospores formed   Later a few disorganised oogonia and antheridia appeared, on egg albumen in 1% potassium phosphate –growth very vigorous with plenty of zoosporangia and chlamydospores, on egg albumen in 1% potassium nitrate -similar growth as above, on egg albumen in 1% asparagin— very little vegetative growth, on boiled house-fly in sterilised water—growth not vigorous, zoosporangia, however, formed, on grams in sterilised water— growth not vigorous, zoosporangia and chlamydospores formed

When a drop of zoospore-suspension was placed on a slide and covered over with a cover slip in an incubator having a temperature of 35° C , the zoospores germinated after 24 hours (Fig 5)

Discussion –This species has already been described by Chaudhuri and Kochhar (1935)[1] when certain variations were observed from the characters given by Coker [2] In the present specimen further variations have been observed which may be summarised as follows –

Highest diameter of the hyphae recorded by Coker is about 37 μ when grown on mushroom-grub, and by Chaudhuri and Kochhar (1935) 18 6 μ and rarely upto 28 μ   But in the present case it was found to be upto 60 μ in rare cases, though usually when grown on egg albumen in water it varied between 29 84-44 76 μ   Zoosporangia are furnished in most cases by an apical papilla   They are club-shaped to globular with all intermediate forms (Figs 1-4)   The size is again variable 508 2-281 μ × 62 68-38 5 μ Coker (1923) gives the diameter as 20-30 μ and Chaudhuri as 27 6-33 8 μ

The secondary zoosporangia are formed by internal proliferation through the primary ones (Figs 3-4)   This point has been noted by Coker as a characteristic feature in *Protoachlya*, and thus differing from

[1] *Proc Ind Acad Sci* , 1935, **2**, No 2
[2] Coker, 1923, *The Saprolegniaceae*

Saprolegnia This character has been found to be the most common feature in the present specimen Cymose branching which has been so amply shown by Coker and Chaudhuri and Kochhar has been found to be of rare occurrence in the present case

The zoospores are diplanetic, formed in several rows, showing great variation in behaviour Coker dealing with the behaviour of the oospore, says, "The behaviour of the spores on emerging is remarkable and very variable The usual behaviour is for some of the spores, perhaps half or a third to swim slowly away on emerging, the other remaining attached to the sporangium mouth and encysting there" The observations made in the present case are quite different All the zoospores of the same zoosporangium behave alike Either all are very active and swim far away from the zoosporangium or they show very slow movement settling down away from the mouth of the zoosporangium In three cases it was noted that the zoospores collected to form irregular clumps at the mouth of the zoosporangium, thus simulating *Achlya* to a certain extent The normal behaviour was however, of active movement, and the zoospores charged out with great rapidity and each one dashed rapidly away All the zoospores were distinctly seen to possess cilia The zoospores are oblong to oval inside the zoosporangium but become spherical outside (Fig 7) The size of the zoospore as given by Chaudhuri and Kochhar is 12 5 13 2 μ while in the present specimen it was 10 0–12 2 μ

Pythiopsis de Bary, 1888

Morphological characters of the genus as given by Coker and noted by the authors are —

Hyphæ slender, much or little branched Sporangia typically short and plump, spherical, oval, pyriform with a distinct apical papilla, or varying to elongated and irregular, primarily borne at the tips of the hyphæ and multiplied from lateral stalks below the older ones to form more or less dense clusters Spores emerging and swimming as in *Saprolegnia* pip-shaped with two apical cilia, sprouting after the first encystment-monoplanetic Gemmæ resembling the sporangia or oogonia formed plentifully, often in chains, producing zoospores after a rest Oogonia borne like sporangia and gemmæ and resembling them in youth, typically spherical, oval or pyriform with unpitted walls, smooth or with a few blunt processes Antheridia short and thick, typically androgynous from the close neighbourhood of the oogonia Eggs one or few (eccentric with a lunate cap of droplets on one side in *Pythiopsis cymosa*, structure doubtful in *Pythiopsis Humphreyana*)
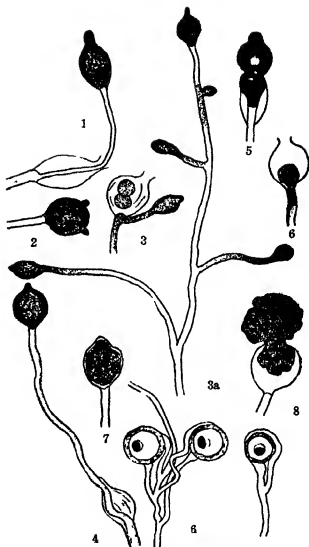
FIGS 1 10 Fig. 1. Internally proliferating zoosporangium Fig. 2 Zoosporangium
with two apical papilla. Fig 3 Cymosely borne zoosporangia 3 *a* Cymosely borne zoo-
sporanga. Fig 4 Internal proliferation of zoosporangium Fig 5 Abnormal internal proli-
feration Fig. 6 Knob formation in internal proliferation. Fig. 7 Zoosporangium half a
minute before the liberation of zoospore mass Fig 8 Zoospore mass coming out in semi-
differentiated state. Fig 9 Hypha with two oogonia Fig. 10. Oogonium with androgynous

P intermedia sp nov (Plate II)

Hyphæ slender, 4 8-5 5 μ in diameter at base, much branched Zoosporangia globular or clavate, usually proliferating internally (Figs 1, 4-6) and borne in a cymose manner A zoosporangium with two apical papillæ has been seen (Fig 2) The secondary zoosporangium has been seen to be growing partly inside and partly outside the empty primary zoosporangium (Fig 5) In cymose branching the secondary zoosporangium is formed either very near the primary zoosporangium or at a fairly long distance away (Figs 3 and 3 a) Zoospores usually 9 5 μ, biciliate, monoplanetic Oogonia plentifully formed in old cultures, spherical, unpitted, 26 6-35 2 μ in diameter (Figs 4-5) Eggs 22 4 28 8 μ in diameter, single, eccentric Antheridial branches long arising far away from the oogonium, one to each oogonium, androgyno is (Figs 9-10), clavate later curving along the oogonial wall Gemmæ resembling zoosporangia formed at the tips of the hyphæ but have a long drawn out apex Gemmæ formed if the mould is kept in stagnant water for a long time or if the acid in the medium is slightly increased

Pythiopsis intermedia sp nov

Hyphæ tenues, basi 4 8-5 5 μ diametro, ramosissimæ Zoosporangia globosa vel clavata, habitualiter interne proliferentia, in cymis disposita Zoosporangia cum duabus papillis apicalibus Aliquando sporangium secundarium ex parte extra et ex parte intra zoosporangium evacuatum crescens Sporangium secundarium vel proxime a primario formatur vel remote Zoosporæ habitualiter 9 5 μ, biflagellatæ, monoplanet* Oogonia rotunda efoveolata, diametro 25 6 35 2 μ, in veteribus culturis abunde occurrentia Ova 22 4-28 8 μ diametro, solitaria, excentrica Rami antheridiales longi, clavati, procul ab oogonio orientes, unus pro unoquoque oogonio, androgyni, post aliquod tempus jexta oogonii parietem incurvantes Gemmæ zoosporangiis similis, in summis hyphis um apicibus longe evectis formatæ Gemmæ habitualiter occurrentes sive in aqua stagnante qua longo tempore hyphæ continentur, sive in medio cujus aciditas leviter augetur

Cultural characters—On egg albumen in tap water—growth vigorous and zoosporangia formed in plenty, in eggs albumen on acidulated water— vegetative growth only, very few zoosporangia formed, on egg albumen in 1% potassium phosphates—abundant zoosporangia formed, on egg albumen in 1% potassium nitrate—vigorous vegetative growth, on egg albumen in 0 1% asparagin—very little vegetative growth, on boiled house-fly in 1% potassium phosphate—vegetative growth vigorous, plenty of zoospo rangia, oogonia and antheridia also formed, on boiled house-fly in tap

water—growth sparse, a few zoosporangia formed, on corn grains in tap water, acidulated water and 1% potassium phosphate—not much growth in any case, on pea grains in tap water, acidulated water and 1% potassium phosphate—results same as above

Zoosporangia make their appearance 30 hours after the placing of new baits After 6 hours all the hyphæ bear zoosporangia A zoosporangium takes 30-45 minutes to discharge the zoospores Soon after liberation of the zoospores, multiplication takes place by either cymose branching or internal proliferation The zoospores swim for 20-25 minutes and then settle down

Cultured from a soil sample taken from a drain in Lahore in November, 1938

Discussion—In this species of *Pythiopsis* the zoosporangia are mostly globular with a dimension of 32 0-41 6 μ (Figs 2, 7) Some are elongated (Figs 1, 4, 5) and their dimensions are usually 31 2 × 23 2 μ or 28 8 × 22 4 μ The hyphæ are much thinner, being 4 8-5 5 μ, while in the other two species (*P cimosa* and *P Humphreyana*) the thickness is much greater

The zoospores come out of the zoosporangium in a semi-differentiated condition by the breaking up of the apical papilla (Figs 1 6) Outside, in the medium, the zoospores are delimited (Fig 8) and each swims away

The size of a zoospore is usually 9 6 μ while in *Pythiopsis cymosa* the zoospores are 8 6-10 8 μ, mostly 9 μ and in *Pythiopsis Humphreyana* they are 8 6 μ Zoosporangia multiply by cymose branching (Figs 3, 3 a) as in other two species and also by internal proliferation (Figs 1, 4, 6) which is not found in the other species of *Pythiopsis*

The oogonium measures 25 6-35 2 μ In *Pythiopsis cymosa* the oogonium measures 18-30 μ, while in *Pythiopsis Humphreyana* 33-89 μ, average being 43 μ The oogonium in this new species contains a single egg measuring 22 4-28 8 μ (Figs 8-9) The egg in *Pythiopsis cymosa* measures 14 8-18 5 μ and in *Pythiopsis Humphreyana* 24-40 μ (average 30 μ) The egg is eccentric as in *Pythiopsis intermedia* and contains a single drop of oil whereas in the egg of *Pythiopsis cymosa* there is a lunate cap of oil droplets The wall of the oogonium is unpitted as in the other two species but the thickness is 3 2 μ in contrast to 1 4-2 μ of *Pythiopsis Humphreyana*

As this species differs in above-mentioned characters from the only two species of *Pythiopsis* the authors have described it as a new species

The most important point is the internal proliferation of the zoosporangia, a character not to be found in either *Pythiopsis cymosa* or *Pythiopsis*
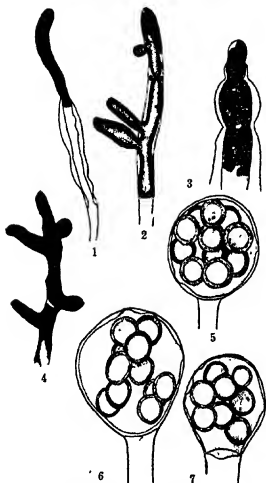
PLATE III   *Saprolegnia rhetica*

FIGS 1–7 Fig 1 Internal proliferation of zoosporangium ×105 Fig 2 Secondary zoosporangium branching ×105 Fig 3 A portion of mature zoosporangium × 487 Fig. 4 Complicated gemma ×105 Figs 5–6 Oogonia with 12 eggs ×487 Fig 7 Oogonium with 9 eggs. ×487

Humphreyana Differences are also found in such other structures as the hypha, zoosporangium, oogonium, egg and its wall and also the position of the oil drop Thus it is named as *Pythiopsis intermedia*, because the size of the zoospores, oogonia and eggs is more or less intermediate between the two species already described

Saprolegnia rhetica Maurizio (Plate III)

Hyphæ branched, 30 4 μ thick Zoosporangia are at times branched and the branches come out of the empty primary zoosporangia (Fig 2) Zoospores 8–9 6 μ in diameter Gemmæ formed, complicated (Fig 4)

Oogonia 72 × 61 6 μ containing 9–12 eggs, generally 12, 19 2 μ in diameter Oogonial wall not very thick, or pitted, very few pits (usually 2–3) (Figs 5–7) Antheridia absent

Growth in culture —On house-fly in tap water—growth extensive, reaching a length of 1 5 cm , large number of zoosporangia formed, eggs develop later, on boiled house-fly in 1% potassium phosphate—vegetative growth extensive, zoosporangia formed but no oogonia, on egg albumen in tap water—vegetative growth vigorous, plenty zoosporangia but on egg albumen in 1% asparagin, very little growth

The present specimen of *Saprolegnia rhetica* resembles the other two identical species (Coker, 1923, de Bary), viz , *S torulosa* and *S variabilis* in the absence of antheridia and few pits on the oogonia walls It differs from the descriptions given by the authors (Maurizio, Minden and de Bary) who first created these species, namely, *S rhetica*, *S variabilis* and *S torulosa*, in minor details of measurements of various organs and number of eggs But on the whole the characters of the specimen are common to all the three species now considered to be identical, hence the inclusion of the specimen under the species *S rhetica*

Collected from Lahore in February, 1939

Summary

In this fourth paper of the series on Indian Water Moulds, the authors have recorded and described three water moulds not so far reported from this country of which, one is a new species These are *Protoachlya paradoxa*, *Pythiopsis intermedia* nov sp and *Saprolegnia rhetica* Maurizio

INDIAN WATER MOULDS—V

A New Genus of the *Saprolegniaceae Hamidia* Gen. nov

By H CHAUDHURI

(From the Botany Department, Panjab University, Lahore)

Locality and Isolation

SAMPLES of water with decaying twigs were obtained from two localities, *viz*, Tarn Taran (Dist Amritsar) and Barhamjit (Dist Hoshiarpur) in March 1936, from which the mould was isolated on potato blocks The growth on potato was luxuriant

Morphology of the Fungus

The general morphological characters of the fungus are as under —

The hyphæ are delicate, sparsely septate 1 7–4 4 μ in diameter (Fig 1), upon which oogonial bodies are borne both in cymose and racemose manner (Figs 2–4) Hyphal wall 0 5–0 8 μ thick The long slender hyphæ are generally racemosely branched, can best be seen in the natural condition, as the staining and teasing processes upset the arrangement

The oogonia when borne singly are attached by delicate and long stalks (Figs 5–6), which are extremely fragile and break even by the slightest disturbance These are also borne in cymose clusters (usually in threes) The diameter of the oogonia varies from 22 2–38 7 μ The oogonia mostly contain a single egg (Figs 7–9), which is quite big and prominent filling the whole oogonium The egg has a wall, catches a deep stain as compared with the hyphæ and 18–34 2 μ in diameter The wall of the oogonium is quite smooth. The single large centric or subcentric egg comes out slowly (Figs 8–9) leaving a hyaline and shrunk capsule behind The egg discharge is slow and almost imperceptible and one has to be vigilant to watch the gradual emergence of the egg Germination of egg while still inside the oogonium has been seen (Fig 19)

It has been noticed that in bodies similar to oogonia, diameter 18–25 5 μ, which may be termed sporangia, 2–7 swarm spores, diameter 6 8–9 5 μ, may be formed (Figs 11–13, 14) These swarm spores are non-ciliate and germinate immediately on being discharged (monoplanetic)

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(Figs 16-17) Occasionally a discharge tube is formed (Figs 14, 15) The swarm spores, when they come out, have no properly differentiated wall If swarm spores are not discharged, then they germinate inside the mother wall and send out germ tubes (Figs 11-13) These swarm spores either germinate by a single unilateral germ tube or produce tubes bilaterally Occasionally it has been seen that only a part of the sporangium forms 1 or 2 spores and the rest germinates by a long germ tube (Fig 18)

Growth in Culture

The fungus was isolated from the decaying twigs under water, and the growth on fresh potato blocks was luxuriant This was the only thing upon which the fungus could be cultured and leucine (1%) had tonic effect Prolonged culture in leucine (1%) and slightly lower temperature about 20° C, produced certain abnormal types of gemmæ (Figs 20-21) though not in abundance Insect and egg yolk were found to be unsuitable The fungus was very sensitive to high temperature and the mycelium lost its vegetative activity soon

Discussion

The word " oogonium " has been used here for the rounded bodies attached to hyphæ due to the close resemblances of the structure of these bodies to oogonium and other characters of the egg Here, as in *Isoachlya*, no antheridia are present The mere absence of the male structures in the neighbourhood of the female cells does not debar one from calling these structures oogonia, so long as these function as such Thus these round cells, with a single egg, can easily be regarded as oogonia

The main characteristics of the fungus, viz (a) Septate hyphæ, (b) Presence of oogonial bodies, (c) Single egg in an oogonium, (d) Absence of antheridia, (e) Presence of swarm spores, and (f) Gemmæ formation, no doubt show Saprolegnialian affinities The mycelium is septate, but the septa being sparse, one is apt to take it as a non-septate cœnocytic mycelium The simple septation is seen in *Blastocladiaceæ* but the absence of the joints and ciliated gametes are radical differences The fungus suggests diverse affinities by its markedly striking characters The delicacy of the mycelium brings it closer to *Monoblepharidales* but the absence of antheridia altogether, is again a primary difference

The formation of swarm spores, the eggs and the gemmæ, are characters of the *Saprolegniaceæ*, though, no doubt, the absence of the true antheridia is a handicap in the proper location of the form Considering, however, all its characters, one feels justified in placing it as a new genus f *Saprolegniaceæ*

Diagnosis

Hamidia Gen nov

Hyphæ hyaline, generally racemosely branched, delicate sparsely septate, bearing oogonia and sporangia both in racemose and cymose manner, best seen in natural condition Oogonia borne singly or in clusters (of usually 3) Oogonia when borne singly have long stalks which break at the slightest disturbance Oogonium with a single large egg entirely filling it Egg with smooth wall, emerges gradually and imperceptibly Apandrous sporangia resembling oogonia also formed, each with 2-7 swarm spores Discharge tube may or may not be present Swarm spores, without a properly differentiated wall and non-ciliate (monoplanetic) germinate soon after discharge Undischarged swarm spores germinate inside the sporangium, putting forth unilaterally or bilaterally produced germ tubes Gammæ also formed Growth on fresh potato stalks most luxuriant, egg yolks and insects unsuitable for growth

Hamidia Gen nov

Hyphæ hyalinæ, communiter racemose ramificantes, tenues, sparse septatæ, oogonia et sporangia tum racemose tum cymose ferentes, quam optime observari possunt in vivo Oogonia singulatim vel acervatim (præsertim ternatum) feruntur Oogonia singulatim producta longis insident pedicellis, qui facillime rumpuntur Oogonia uno tantum magno ovo complentur Ovum, terso pariete, gradatim et sensim sine sensu emergit Fungus est apandrus Sporangia etiam, oogoniis similia, producuntur, quorum unumquodque 2-7 zoosporas habet Tubulus quo sporæ liberantur potest vel adesse vel abesse Zoosporæ pariete non plene differentiato et non ciliato (monoplano) germinant cito post liberationem, zoosporæ quæ non liberantur, germinant in sporangio, unilateraliter vel bilateraliter productis germinationibus tubulis Gemmæ etiam producuntur Crescit luculentissime super vivos truncos Solani tuberosi, non crescit in medio ex ovorum vitellis vel in insectis
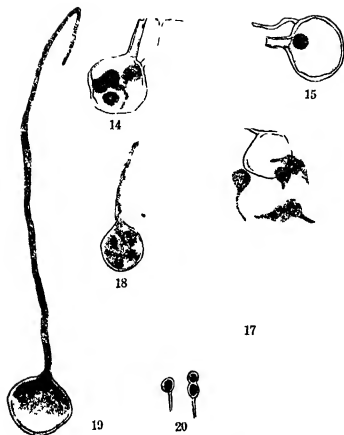
Hamidia indica sp nov. (Plate I)

Characters as above Hyphæ 1 7-4 4μ broad Oogonia wall smooth Oogonia 22 2-38 7μ in diameter, eggs 14-34 2μ in diameter Sporangia 18-28 9μ in diameter On decaying twigs under water Collected by A. Hamid in March 1936

Hamidia indica sp nov

Characterse ut supra Hyphæ 1 7-4 4μ latæ Oogonii parietes tersi, oogonia 22 2-38 7μ diam, ova 14-34 2μ diam, sporangia 18-28 9μ
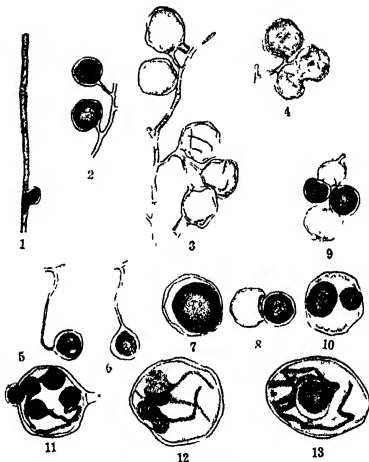
PLATE I   *Ramidia indica*

FIGS 1–13 Fig 1 Vegetative hyphæ ×636 Fig 2 Arrangement of oogonia ×525
Fig 3 Arrangement of oogonia and sporangia ×600 Fig 4 Arrangement of oogonia
×525 Figs. 5–6 Oogonia with elongated stalk ×600 Fig 7 One large egg before
emergence ×600 Fig 8–9 Various stages during the emergence of egg ×525 Fig 10
Two eggs formed in an oogonium ×600 Figs. 11–13 Swarm spores germinating inside the
mother cell (sporangium) ×600.

FIGS 14–21 Fig 14 Spores germinating inside a stalked sporangium ×600 Fig 15 A swarm spore just before escaping from the sporangium through a discharge tube ×600 Figs 16–17 Swarm spores germinating outside the sporangium ×600 Fig 18 Sporangium partly forming spores and partly germinating into vegetative hypha ×600 Fig 19 Egg germinating. ×600 Figs 20–21 Gemmae ×525

diam. Occurrit sub aqua super putrescentes ramusculos arborum. Collectus ab A Hamid, mense martio 1936

Type specimens deposited in Panjab University, Botany Department, Lahore

Summary

A new genus of *Saprolegniaceæ*—*Hamidia* has been isolated, grown in culture, and described, and a new species *Hamidia indica* established The Latin diagnosis in both cases has been given

The author expresses his thanks to Dr B B Mundkur for kindly looking through the manuscript and making valuable suggestions and to Rev Father Rapinat and Prof H Santapau for the Latin diagnosis

CONTRIBUTIONS TO THE BIONOMICS, ANATOMY, REPRODUCTION AND DEVELOPMENT OF THE INDIAN HOUSE-GECKO, *HEMIDACTYLUS FLAVIVIRIDIS* RÜPPEL

Part III   The Heart and the Venous System

By BENI CHARAN MAHENDRA

(Department of Zoology, St John's College, Agra)

CONTENTS

I   Introduction

ALTHOUGH it is more than fifty years ago that Hoffmann (1890, p 1010) complained about the paucity of detailed accounts of the venous system in Lizards—a complaint repeated in 1920 by O'Donoghue—we have still

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not much knowledge about this system in a great many families of Sauria In particular, the deficiency of such information about the family Gekkonidæ is to be deplored, as this family approaches the Rhynchocephalia such a great deal in its organization The present account aims to fill this gap in our knowledge, as well as to complete the description of the vascular system of *Hemidactylus flaviviridis*, which Bhatia and Dayal (1933) started with the excellent paper on its arterial system nine years ago

To mention the more important contributions on the subject, Corti (1853) described the vascular system in "*Psammosaurus griseus*" (*Varanus griseus*), while Brücke (1852), Rathke (1857) and Fritsch (1869) gave valuable accounts of the heart and aortic roots in a number of lizards   Hoffmann (1890) and Hochstetter (1893) *inter alia* and Grosser and Brezina (1895) particularly dealt with the development of the veins in *Lacerta*   Greil (1903) studied the structure and development of the heart in *Lacerta* and some other lizards Beddard (1904-06) made valuable observations on the veins of numerous lizards, such as *Heloderma suspectum, Varanus griseus, V niloticus, V exanthematicus, Iguana tuberculata, Tiliqua scincoides, Tupinambis, Chamæleon, Pygopus lepidopus, Phelsuma madagascariensis, Tarentola annularis, Gerrhosaurus flavigularis, Ophisaurus apus,* and *Amphisbæna brasiliana* Bruner (1907) described, in connection with the cephalic vessels, a muscular mechanism for raising the blood pressure in the head Goodrich (1916, 1919) pointed out the phylogenetic importance of " the subdivision of the aortic trunk so as to form two systemic arches crossing at their base in such a way as to become separated by the interventricular septum ' O'Donoghue (1918) noted the condition of the septum ventriculorum and its relation to the openings of the aortic arches in various reptiles and criticised Goodrich Bhattacharya (1921) and Thapar (1921) described the venous system in *Varanus bengalensis* (now *V monitor*) John (1924) proved that the flow of blood in *Varanus monitor*, contrary to the generally accepted belief, is from the Hepatic Portal to the Renal Portal System by means of the short cross-connection Bhatia (1929) described the heart and the venous system of *Uromastix hardwickii* in detail Finally, Benninghoff (1938) gave an excellent résumé of our knowledge of the heart, and Gelderen (1933), that of the venous system in the whole Vertebrate series

2 Technique

The structure of the heart was studied in thick hand-cut sections (both transverse and horizontal) of formalin-preserved material, in careful dissections, and in serial sections, 15 μ thick, prepared according to the paraffinembedding process and stained with borax carmin and hæmatoxylin,

Most of the veins can be made out, even without injection, in a freshly captured and killed specimen. Geckos, kept for a long time in captivity, lose much of their blood and are not so good for demonstration of the venous system, unless injected. Beale's Prussian Blue (Lee's *Microtomist's Vade Mecum*, 1928, p 248) and Emery's Aqueous Carmine (*ibid.*, p 247) were successfully tried, although the best results were obtained by the injection of Reeve's Fixed Indian Ink.

3 *The Heart*

The heart in *Hemidactylus flaviviridis* lies in the mid-ventral line between the bases of the fore-limbs immediately behind the neck. Such a forward position is worth noting, as it perhaps represents a primitive condition. As shown by Rathke (1857), the farther back does the heart lie in the pleuroperitoneal cavity, the more highly organised is the reptile. The heart (Text-Fig. 1) is almost as broad as long, being about 6 mm in width at its anterior end in a full-grown individual. It is roughly conical in appearance, the base of the cone being directed anteriorly and the apex posteriorly. The
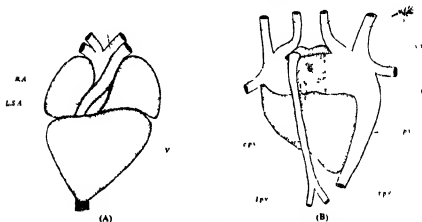
Text-Fig 1

The heart of *Hemidactylus flaviviridis* (A) Ventral view (B) Dorsal view.

a v c, anterior vena cava (left); *C C D*, right common carotid arch; *C C S*, left common carotid arch; *c p v*, common pulmonary vein; *d*, fluted region of the sinus venosus; *G c* gubernaculum cordis; *l p v*, left pulmonary vein; *L S A*, left systemic trunk; *L A*, left auricle; *P A*, pulmonary arch; *p v c*, posterior vena cava; *R A*, right auricle; *r p v*, right pulmonary vein; *R S A*, right systemic arch; *v j c*, common jugular vein; *s v*, subclavian vein; *v t*, tracheal vein; *V*, ventricle.

apex is obtusely rounded and is tied to the posterior pericardial wall by a ligamentous band, the *Ligamentum apicis cordis* or *Gubernaculum cordis*

Internally, the heart shows the typical saurian structure Its cavity is divisible into a thin-walled sac on the dorsal side (the *sinus venosus*), two antero-lateral chambers (the *right* and *left auricles*), and a conical thick-walled sac (the *ventricle*) The *conus arteriosus* is absent as a distinct chamber, having been absorbed in the ventricle *

(A) *Sinus Venosus*

The sinus venosus (Text-Fig 1, B) is a dorso-ventrally flattened chamber and lies transversely across the part of the auricles directly in front of the anterior border of the ventricle It is formed as in other lizards, by the confluence of the two *anterior vena cava* with the *posterior vena cava*, but it shows several peculiarities Its right half is somewhat larger than the left, being formed by the union of the right anterior vena cava with the posterior, the latter sweeping forwards distinctly to the right of the heart before opening into the sinus venosus There is no internal ridge (*tuberculum intervenosum*), corresponding to that in the Mammalia, between the opening of the right anterior vena cava and that of the posterior The trachea in *Hemidactylus flaviviridis* lies closely adpressed to the medio-longitudinal part of the sinus venosus, and consequently all this region of the sinus, when empty, is strongly fluted a characteristic which has not been so far recorded in any other reptile Bhatia (1929) noted a slight median constriction between the right and left portions of the sinus venosus in *Uromastix hardwickii*, but the characteristic groove-like depression in *Hemidactylus*, which evidently serves for the accommodation of the trachea, cannot be compared with it, there is no constriction of the sinus venosus in *Hemidactylus*, comparable to that shown by Bhatia in his diagram of the heart of *Uromastix*

The *sinu-auricular aperture* is a narrow semi-circular slit in the dorsal wall of the middle portion of the sinus venosus, lying at right angles to the antero-posterior axis of the heart Its lips, like those in *Uromastix hardwickii* (Bhatia, 1929) are valvular, there being, however, no distinct valves as described by O'Donoghue (1920) in *Sphenodon punctatus*

(B) *Atrium Dextrum* (*The Right Auricle*)

The right auricle is slightly larger than the left In *Sphenodon punctatus* (O'Donoghue, 1920), *Uromastix hardwickii* (Bhatia, 1929), and a snake

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* Benninghoff (1933) has given an account of the manner in which the *conus arteriosus* (*Bulbus*) is believed to have been absorbed in the ventricle

Ptyas mucosus (Ray, 1934), a small sac-like diverticulum has been described, arising from its antero dorsal mesial edge I do not find such a structure in the heart of *Hemidactylus flaviviridis*

(C) *Atrium Sinistrum (The Left Auricle)*

The left auricle, although slightly smaller than the right, resembles the latter in its general appearance Its postero-ventral portion, however, especially when full of blood, projects backwards over the left antero ventral border of the ventricle to a considerable extent The common pulmonary vein opens in the dorsal wall of its posterior portion near the inter-auricular septum

(D) *Ventriculus*

The ventricle is a stout-walled, slightly asymmetric, conical sac with its apex directed backwards, and is distinctly marked off from the auricles by a deep *coronary sulcus* Its right lateral surface is evenly convex, while its left one shows a slight concavity

A study of serial transverse sections show four successive regions in the ventricle, passing gradually and insensibly into each other As these regions have not been so far recognised or described in detail [the only other account of the saurian heart, based on a reconstruction of serial sections, being by Rau (1924) who has more particularly studied the origin of the aortic arches in *Tiliqua scincoides*], I give a concise account of these regions here

In the first place, there is the hindmost, *apical portion* of the ventricle (Text-Fig 2), characterised by the presence of numerous *muscular trabeculae*,
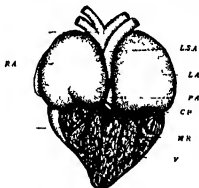

TEXT-FIG 2

Transverse section passing through the apical part of the ventricle

lac, lacunae *m t*, muscular trabeculae

dividing the internal cavity into a series of irregular lacunae An important point about this region, not previously observed, is, that the trabeculae (at least the vast majority of them) extend in a dorso-ventral direction, so that the ventricular spaces are in the form of laterally compressed, vertical chambers This would naturally result in the separation of the blood on the right side

of the ventricle from that on the left, while permitting a flow of both kinds from the dorsal portion of the ventricle, in which the auricles open, into the ventral portion, from which the aortic arches take their origin (*vide infra*)

Secondly, there is—immediately preceding the apical region—the region of *origin of the muscular ridge* This ridge, called " Muskelleiste " by Greil and other German workers, has been identified by Goodrich (1916, 1919, 1930), O'Donoghue (1918) and Rau (1924) as the *septum ventriculorum* As I do not intend to commit myself to the latter determination, which should be substantiated by a reference to development,* I prefer to call the structure " the muscular ridge '

The *muscular ridge* in *Hemidactylus flaviviridis* (Text-Fig 3) arises as a prominent structure on the ventral wall of the ventricle distinctly towards
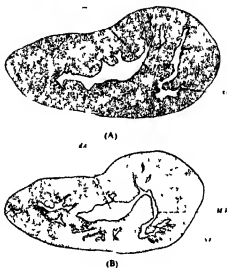

TEXT-FIG 3

Ventricle dissected from the ventral side to show the free edge (near *sl*) of the muscular ridge
C P, cavum pulmonale, *M R* muscular ridge *R S* right systemocarotid arch *Sl* slit near the free border of the muscular ridge (other abbreviations as in Text-Fig 1)

the right and is, at its origin, obliquely vertical (Text-Fig 4, A) As it passes forwards it becomes more and more inclined into a horizontal direction, and thus separates a small *ventro-lateral* cavity on the right side from a *dorso-lateral* one on the left (Text-Fig 4, B) The former is the " *cavum pulmonale* ", while the latter corresponds both to the ' *cavum venosum* " and " *cavum aorticum* " together A new feature in the ventricle of *Hemidactylus flaviviridis* is the presence in addition to the muscular ridge, described above, of another ridge, shorter and more slender, arising from

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* Both Greil a and Hochstetter s work on the development of the heart seem to be against Goodrich s determination

(A)

(B)

TEXT-FIG 4

Transverse sections through the ventricle, showing the muscular ridge
Section A is caudad to B

d, dorso lateral chamber *M R*, muscular ridge *s r*, supplementary ridge

the dorso-lateral wall of the ventricle towards the right of the muscular ridge and running in a direction opposite to the latter As far as I know, no previous author has recorded this structure This additional ridge obviously supplements the muscular ridge

Thirdly, there is the region of the *auricular apertures* (Text-Fig 5), which both lie dorso-lateral to the muscular ridge and are separated from each other by an obliquely vertical septum

TEXT-FIG 5

Transverse section of the ventricle in front of that figured in Text-Fig 4 B

L au ap, left auriculo-ventricular aperture, *r.av ap*, right auriculo-ventricular aperture.

Fourthly, there is the anterior region (Text-Fig 6) of the ventricle, from which the aortic arches take their origin It is interesting to note that
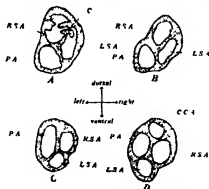

TEXT-FIG 6

Transverse section of the ventricle caudal to the definitive origin of the aortic arches
a M R , anterior extension of the muscular ridge Other abbreviations as in previous figures

the openings of all the aortic arches are situated at the anterior extension of the free border of the muscular ridge, and thus appear to lie, at different levels, in the "cavum pulmonale"

(E) *Trunci Arteriosi*

On account of the absorption of the conus arteriosus, the three aortic arches take their origin directly from the lumen of the ventricle Of the three, the pulmonary arch arises most posteriorly, as a forward prolongation of the "cavum pulmonale", and lies at its origin latero-ventrad to the left of the left systemic arch (Text-Fig 7) The two systemics can be

TEXT-FIG 7

Successive transverse sections showing the shifting positions of the aortic trunks.
Section A is the most caudad

C , point of intercommunication of the systemic trunks, *C C A* , common carotid arch,
L S A , left systemic arch, *P A* , pulmonary arch, *R S A* , right systemic arch

traced backwards to a point slightly anterior to the point of origin of the pulmonary arch, where they intercommunicate with each other. At this point, the right systemic arch lies dorsally to the other two arches, while the left one lies below it, slightly dorsal and distinctly to the right of the pulmonary arch

Soon after their origin the aortic arches shift their positions in relation to each other. The pulmonary trunk gradually assumes a dorsal position. The left aortic arch shifts from its origin on the right side, at first, to a distinctly ventral position, and then towards the left side. The right aortic arch passes over from its original dorsal position to a position on the right side. The carotid trunk is cut off from the right systemic arch towards its dorsal aspect slightly towards the left, as the latter is descending to assume its proper position.

1 The Anterior Vena Cava

In the present description of the venous system of *Hemidactylus flavi-viridis* I have followed, as far as possible, the manner and arrangement so ably adopted by O'Donoghue (1920) in his exposition of the vascular system of *Sphenodon*. The veins are described from the heart towards the periphery and a consistent system of numbering them with alphabets, sometimes coupled with Roman numerals, is used. As the two anterior venae cavae are similar in their disposition, only the left is described in detail.

The left anterior vena cava (*Vena cava anterior sinistra*) is an extremely short stout trunk passing backwards and inwards to open into the left anterior border of the sinus venosus (Text-Fig 8). It is formed, immediately outside the pericardium, by the union of three veins—the *Vena trachealis* (A), the *Vena jugularis communis* (B), and the *Vena subclavia* (C). Although there may be slight variations in the mode of union of these three veins in a vast majority of individuals, the former two trunks definitely unite together before they are joined by the subclavian. This is clearly a point of difference from *Sphenodon* in which all the three trunks are figured by O'Donoghue as meeting at the same place (O'Donoghue's Text-Fig 7). In "*Psammosaurus griseus*" the anterior vena cava, according to Corti (1853), is formed by the union of two vessels, *Vena jugularis* and *V subclavia*, the former of which might perhaps be regarded as representing the conjoined *V trachealis* and *V jugularis communis* of *Hemidactylus*. A similar condition is shown in a figure of *Varanus monitor* by Thapar (1921), although his terminology at places is different from the one used by O'Donoghue (1920)
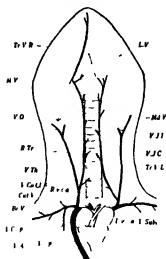
TEXT FIG 8

The anterior venae cavae and their tributaries

Br V, vena brachialis *Cut V*, cutaneous vein *LV* vena lingualis, *L v c a* left anterior vena cava, *M V* ramus from muscles, *Md V* vena mandibularis *R Tr* rami tracheales *R v c a*, right anterior vena cava, *Tr V L*, left tracheal vein *Tr V R* right tracheal vein *V Az* vena azygos, *V J C*, vena jugularis communis *V J I* vena jugularis interna *V O* venæ œsophageæ *V Th* vena thyreoidea *V c p*, vena cava posterior, *V Cœ Cl* vena coraco-clavicularis *V C o p* vena coraco-pectoralis *V Subc* vena subclavia

(A) *Vena trachealis*

The *Vena trachealis* (Text-Fig 8), traced cephalad from the point of its junction with the *Vena jugularis communis*, runs forwards dorsad to the carotid and systemic arches, turning slightly to the middle line so as to approach the lateral surface of the trachea It then extends forwards lateral and parallel to the trachea, receiving minute tributaries from it, as well as from the thyroid gland (*Vena thyreoidea*), the œsophagus (*Venæ œsophageæ*) and the adjoining muscles (*Rami musculares*) ˙ After passing dorsal to one of the posterior arms of the basihyoid, it receives one or two small factors from the larynx (*Vena laryngeæ*), as well as a few from the ventral wall of the œsophagus (*Venæ œsophageæ*)

The left tracheal vein, like that in *Sphenodon* (O'Donoghue, 1920), is smaller than the right It begins approximately at the level of the larynx, while the right one is a stout vessel extending back right from the mandibular symphysis Anterior to the place where the left tracheal arises, the right

tracheal sweeps gradually inwards so as to occupy a mesial position It also receives a vein from the tongue (*Vena lingualis*)

In *Sphenodon punctatus* (O Donoghue, 1920) some of the laryngeal tributaries of the tracheal veins anastomose with each other to produce a venous network, and in *Varanus monitor* (Thapar, 1921) there are four anastomoses between the right and the left tracheals I find no such connections in *Hemidactylus flavivirıdis*

As pointed out by O'Donoghue (1920), the tracheal vein is erroneously termed the *external jugular* by Parker (1884), a usage which was adopted by Thapar (1921) in his paper on *Varanus monitor* The vein in question, runs *dorsad* to the arterial arches and cannot be homologised with the external jugular in the Amphibia, which latter runs *ventrad* to them

In *Lacerta*, the left tracheal vein is absent in the adult, although it is represented in the embryos (Bruner, 1907, Grosser and Brezina, 1895) During development, however, anastomoses arise between the two tracheals, so that the right tracheal gradually takes upon itself to drain the left side also, this leading ultimately to the entire disappearance of the left tracheal vein O Donoghue (1920) claims that the condition in *Sphenodon* is primitive as it represents the stage in *Lacerta* when anastomoses have appeared between the two tracheal veins The same might perhaps be said for *Varanus monitor* If such claims are tenable, the condition in *Hemidactylus flavivirıdis*, where there are two tracheal veins with practically no anastomoses between them, might be regarded as very primitive

(B) *Vena jugularis communis*

The *Vena jugularis communis* traced cephalad, runs slightly upwards from the point of its union with the *Vena trachealis*, extends forwards laterally in the neck and receives small veins from the adjacent muscles The part of the common jugular vein extending in the neck, strictly speaking, corresponds to the *Vena jugularis interna* of Grosser and Brezina (1895), Bruner (1907) and O'Donoghue (1920) while the *external jugular* vein must be regarded either as altogether absent, or perhaps as represented by one of the tributaries from muscles, which open into the *Vena jugularis communis* in its post-cervical part

The *vena jugularis interna* receives, just behind the angle of the jaws, a large vein, the *Vena mandibularis* (*Vena maxillaris inferior*, Grosser and Brezina) from the lower jaw and its muscles In front of this point, its main stem extends on the ventral aspect of the cranium and receives factors from the brain, palate and other parts of the head As shown by the developmental studies of Grosser and Brezina on *Lacerta* and *Tropidonotus* (1895),

this stem consists of three parts (a) the posterior part formed from the anterior cardinal vein, (b) the middle part, derived from the vena capitis lateralis, and (c) the anterior part, representing the remnant of the vena capitis medialis and the orbital sinus

(C) *Vena subclavia*

The subclavian vein is a stout vessel bringing blood from the fore-limb, shoulder girdle and the ventral and dorsal body wall Traced distally from the point of its union with the jugular and tracheal veins, it runs straight outwards towards the base of the fore-limb, receiving a *Vena coraco-clavicularis* from the dorsal part of the shoulder girdle, a *Vena azygos* from the costal and vertebral regions, a *Vena coraco-pectoralis* from the ventral region of the shoulder girdle and some factors from the skin The main trunk extends into the upper arm as the *Vena brachialis* The formation of the subclavian vein thus resembles that in *Sphenodon punctatus* considerably
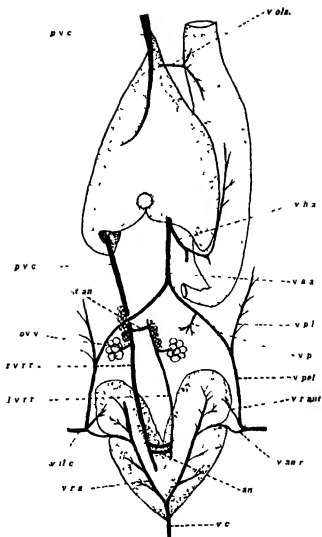
5   *Vena Cava Posterior*

The *posterior vena cava* (Text-Fig 9) is a large thin-walled vessel, arising by the union of the two *venæ renales revehentes* (A) at the level of the epididymes in the male and at the corresponding region in the female It lies rather to the right of the mid-longitudinal line, enters the substance of the liver at the ventral aspect of the dorso-posterior prolongation of the right lobe and comes out ventro-mesially a little behind the attenuated anterior edge of this gland It receives a number of minute *hepatic veins* (B) during its passage inside the liver, and on coming out of that organ, sweeps round to the right side of the heart to open into the postero-lateral part of the sinus venosus

(A) *Venæ renales revehentes*

The *venæ renales revehentes* (*Efferent Renal Veins*), which drain the blood brought to the kidneys, take their origin in Sauria (as far as hitherto recorded) in three ways

(1) *Post-renal Origin*—Both Thapar (1921) and Bhattacharya (1921) observed that the *venæ renales revehentes* in *Varanus monitor*, instead of taking their origin entirely from the substance of the kidneys, appear to arise behind them in the form of a post-renal transverse vessel, which (according to Bhattacharya) even receives a small vein from the dorsal region of the rectum A condition somewhat similar to it was described by Beddard (1904) in *Pygopus lepidopus* in which " the efferent renals arise at first as a single trunk very near to the posterior end of the kidneys,

Text-Fig 9
The posterior veins

a n , anastomoses between venæ renales revehentes , *l v r r* and *r v r r* , right and left venæ renales revehentes *o v v* ovarian vein , *p v c* , posterior vena cava , *t a n* , transverse anastomosis , *v a a* , vena abdominalis anterior , *v a n r* , vena anastomotica renalis , *v c* , vena caudalis , *v h a* , vena hepatica advehens , *v il c* , vena iliaca communis , *v æ s* , vena œsophagus , *v p* , vena pubica , *v p l* , vena parietalis lateralis , *v pel* , vena pelvica , *v r a* , vena renalis advehens , *v r-ant* , vena renalis anterior

and of course between them This trunk divides into two before reaching the middle of the kidneys " (see also Beddard's text-fig 4)

(2) *Intra-renal Origin* —In *Lacerta* (Hochstetter, 1893), the *venæ renales revehentes* take their origin separately in the kidneys, but are connected to each other by a small anastomosis near the posterior end of that organ

(3) *Sinusoidal Origin* —Bhatia (1929) found a large sinus in the middle region of the conjoined kidneys in *Uromastix hardwickii* The sinus receives several factors from the substance of the kidneys and gives rise anteriorly to the two *venæ renales revehentes*

In *Hemidactylus flaviviridis*, the *venæ renales revehentes* arise in the second of the foregoing three ways They commence, by the confluence of minute efferent renal factors, towards the posterior attenuated ends of the kidneys and run forwards on the ventral aspect close to the mesial border of these organs, separated from each other by the dorsal mesentery The right vein, as usual in most Sauria and in *Sphenodon*, is distinctly larger than the left Unlike *Lacerta* however, in which there is only one inter-renal anastomosis, and *Sphenodon* in which there are four such connections between the *venæ renales revehentes*, the veins in *Hemidactylus flaviviridis* are connected to each other by two (rarely one) anastomoses

Each *vena renalis revehens* runs forwards in front of the kidney, receiving some minute tributaries from the mesentery, and comes to lie along the mesial border of the supra-renal organ of its side The left vein finishes here, it discharges into the right *vena renalis revehens* by a transverse anastomosis The right vein, from this point forwards, extends as the *posterior vena cava* to open into the liver The arrangement in *Hemidactylus flaviviridis* thus closely resembles that found in *Lacerta* (Parker's fig 40, 1884), *Uromastix hardwickii* (Bhatia, 1929) and *Sphenodon* (O'Donoghue, 1920), but it differs from that in *Varanus monitor*, in which (as ascertained by me by dissection) the two *venæ renales revehentes*, although obliquely directed from the median line towards the right, are of the same calibre and contribute equally to the formation of the *posterior vena cava*

The *venæ renales revehentes* receive the spermatic (A I), ovarian, and supra-renal (A II) veins

A I *Venæ spermaticæ* —The *venæ spermaticæ*, which of course are present only in the male, drain the blood of the testes and mesorchia As in *Sphenodon* (O'Donoghue, 1920), the left spermatic vein opens into the left *vena renalis revehens*, while the right one opens into the posterior vena cava in front of the latter's junction with the transverse anastomosis from the

left efferent renal. This is different from the condition found either in *Uromastix hardwicku* or in *Varanus monitor*. In *Uromastix*, as figured by Bhatia (1929), the right testis is supplied with four *venæ spermaticæ*, two of which open into the transverse anastomosis and the rest into the posterior vena cava. In *Varanus monitor*, although Thapar (1921) and Bhattacharya (1921) do not describe the arrangement, I find that the right *vena spermatica* opens not into the posterior vena cava but into the *vena renalis revehens* of its side. The disposition of the *ovarian veins* is similar to that of the spermatic but the right ovarian is situated anterior to the left.

A. II *Venæ supra-renales revehentes*—O'Donoghue (1920) found that in *Sphenodon* these veins, a series of minute twigs, open into the vena spermatica of their side. In *Uromastix hardwicku*, according to Bhatia (1929), each supra-renal body sends a supra-renal vein to meet the spermatic of its side. Neither Thapar (1921) nor Bhattacharya (1921) mention anything in this connection in *Varanus monitor*. In *Hemidactylus flaviviridis* there are several very minute supra-renal efferent veins corresponding apparently to those found in *Sphenodon*, but they open not into the vena spermatica as in that reptile but directly into the vena renalis revehens adjacent to them.

A. III *Venæ oviducales*—In the female, a number of minute oviducal veins can be traced running across the broad ligament to open into the vena renalis revehens. The disposition of these veins thus represents an extremely simplified condition different from that described in *Iguana tuberculata* (Beddard, 1904), *Chamæleon vulgaris* (Beddard 1904), *Pygopus lepidopus* (Beddard, 1904), and *Heloderma suspectum* (Beddard, 1906).

6 *The Supra-renal Portal System*

To quote from O'Donoghue (1920), " the presence of this system has already been noted in the *Varanidæ* by Corti, Hochstetter and Beddard, in which genus it is very well developed. It is subject to a great deal of variation in the Lacertilia, as Beddard has shown, and although usually present and often well marked, as in the *Varanidæ, Iguana tuberculata, Ophisaurus apus* and *Amphisbæna brasiliana*, it may be considerably reduced, e.g., *Tiliqua scincoides*, or even absent altogether, save as an abnormal

variation, as in *Chamæleon vulgaris* In *Sphenodon* it has apparently retained more primitive relations than in the other forms described, and this throws useful light on the question of its constitution " Its main factor is a vein termed differently by various authors Hochstetter (1893) called it *Vena deferentialis*, while Beddard (1905) termed it the *posterior cardinal vein* O'Donoghue (1920) suggests that it should be called *Vena supra-renalis advehens*

In *Hemidactylus flaviviridis*, I find that the *supra-renal portal system* is absent

7 The System of the Caudal Vein

The *vena caudalis* (Text-Fig 9), as usual in the Sauria, runs mid-longitudinally ventrad to the caudal artery in the tail and receives tributaries from the caudal muscles in each segment Anteriorly, it comes to lie on the mid-ventral aspect of the posterior tapering, conjoined ends of the two kidneys and bifurcates, approximately at the level of the anus, to form the paired *vena renales advehentes* The point of bifurcation of the *vena caudalis* in *Sphenodon punctatus* as described and figured by O'Donoghue (1920), lies differently behind the posterior ends of the kidneys, while in *Uromastix hardwickii*, the *vena caudalis* according to Bhatia (1929), ' enters the kidney where it becomes split up into Y-shaped diverging limbs running forward and becoming partially buried in its substance ' The condition in *Hemidactylus* corresponds to that found in *Uromastix* rather than in *Sphenodon* Close to the point of its bifurcation, the *vena caudalis* receives a pair of *inguinal* veins, bringing in blood from the inguinal region

The two *vena renales advehentes*, unlike those in *Sphenodon*, are equi-sized They extend forwards on the ventral aspect of the kidneys, each receiving a *vena cloacalis* and a *vena rectalis* during its course, and disappearing in the substance of the anterior lobe of the kidney a little behind its anterior border

From each *vena renalis advehens*, almost at its mid-point, a branch runs outwards to the cleft between the anterior and posterior lobes of the kidney, passes upwards in this cleft and joins the *iliac* vein Such an anastomosis occurs also in *Uromastix hardwickii* (Bhatia, 1929), *Sphenodon punctatus* (O'Donoghue, 1920) and *Lacerta* (Hochstetter, 1893, Plate 16, fig 12), being more superficial in the last genus, but this sort of disposition differs conspicuously from that found in *Varanus monitor* (Bhattacharya, 1921), *Pygopus lepidopus* (Beddard, 1904), and *Chamæleon vulgaris* (Hochstetter, 1893, Beddard, 1904)

8 *The Hepatic Portal System*

Although the anterior abdominal and epigastric veins form part of a single system afferent to the liver I follow O Donoghue in treating them separately for the sake of clarity and convenience

(A) *The System of the Anterior Abdominal Vena*

The *Vena abdominalis anterior* runs mid-longitudinally along the ventral body wall and is formed by the union of the paired *venæ pelvicæ* in front of the pelvis It receives a small tributary from the duodenum a little in front of the place of its formation, extends forwards to receive the *Vena hepatica advehens (Vena porta)*, and enters the left lobe of the liver

A *Vena pelvica* The pelvic vein arises as a branch of the *Vena iliaca communis* at the outer aspect of the kidney and extends, from its point of origin, downwards and mesialwards to join its fellow of the other side to form the *vena abdominalis anterior* During its course it receives the following tributaries —

A   I   *Vena parietalis lateralis* (Lateral abdominal vein of Beddard)

It arises as a delicate vessel approximately at the level of the middle of the liver passes backwards in the angle between the dorsal and ventral body wall and receives tributaries from the intercostal muscles It opens into the pelvic vein at the place where the latter is curving downwards towards the mid-ventral line

A   II   *Vena pubica* This is a small vein coming from the ventral aspect of the pelvis near the pubic symphysis

A   III   *Vena vesica* —The vesicular vein comes from the ventral wall of the urinary bladder

B   *Vena iliaca communis* -This vein, called by Hochstetter *Vena ischiadica*, extends from inside the hind-limb towards the outer border of the kidney and divides into three branches --

B   I   *Vena pelvica*, which has been described above

B   II   *Vena renalis anterior* —In *Sphenodon punctatus* (O'Donoghue, 1920), this vein, composed of several twigs from the first kidney lobe," opens into the *vena anastomotica renalis* In *Hemidactylus flaviviridis*, however, I find that it is an independent branch of the *vena iliaca communis*, and is altogether separate from the *vena anastomotica renalis*

B   III   *Vena anastomotica renalis* —This is a vein running from the common iliac into the cleft between the anterior and posterior kidney lobes and joins the *vena renalis advehens*

(B) *The System of the Epigastric Veins*

In *Hemidactylus flaviviridis*, this system is composed of a large trunk, *Vena hepatica advehens* (A), joining the anterior abdominal vein, and a small vein *Vena œsophagea* (B), from the œsophagus and cardiac end of the stomach Besides these, there is a small *parietal vein*, collecting blood from the ventral aspect of the vertebral column and opening into the dorsal side of the right lobe of the liver a little in front of the entry of the posterior vena cava This last has not been recorded previously

A *Vena hepatica advehens* —The formation of this vein in Sauria, as shown by the work of many authors, is subject to a great deal of variation In *Hemidactylus flaviviridis* (Text-Fig 10), it is formed, a short
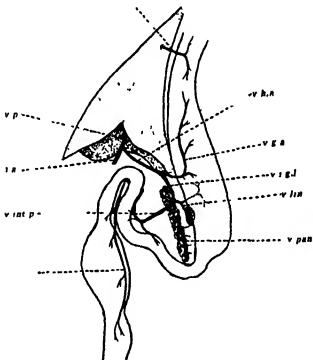
Text-Fig 10

The hepatic portal system

v.a a, anterior abdominal vein, *v g a*, vena gastrica anterior, *v h a*, vena hepatica advehens, *v.i g l*, vena intestino-gastero-lineals, *v.int p*, vena intestinalis posterior, *v lin*, vena linealis, *v.œs*, vena œsophagea, *v p*, vena porta, *v pan*, vena pancreatica, *v.r*, vena rectalis.

distance behind its junction with the anterior abdominal vein, by the union of two factors the *vena gastrica anterior* (A I) and the *Vena intestino-gastero-linealis* (A II)

A I *Vena gastrica anterior*—This is a large vein arising near the anterior end of the stomach and running longitudinally backwards on its wall, receiving a number of minute tributaries Unlike the one in *Sphenodon punctatus* (Beddard, 1905, O'Donoghue, 1920), it has nothing to do with the anterior abdominal vein, not to speak of its being regarded as a continuation of the latter in any sense

A II *Vena intestino-gastero-linealis*—This is a large trunk formed by the union of two veins (i) the *Vena gastero-linealis*, which receives tributaries from the posterior dorsal half of the stomach wall, the spleen and the pancreas, and (ii) the *Vena intestinalis posterior*, which collects blood from the duodenum, ileum, rectum and cloaca

B *Vena œsophagea* The œsophageal vein arises by the union of an anterior with a posterior tributary on the wall of the œsophagus and cardiac region of the stomach It runs in the gastro-hepatic omentum to open into the tapering anterior end of the liver towards the left of the emerging posterior vena cava Although not described in *Sphenodon punctatus*, it occurs in *Uromastix hardwickii* (Bhatia, 1929) and seems to correspond with the *gastro-hepatic vein* in *Varanus monitor* (Thapar, 1921), *Varanus griseus* (Hochstetter, 1893), *Varanus exanthematicus* and *Varanus niloticus* (Beddard, 1906) Beddard (1906) laid stress on its limitation to a single vein in the genus *Varanus* as a noteworthy difference from many other Lacertilia The same condition, however, was reported by him in the two geckos, *Phelsuma madagascariensis* and *Tarentola annularus* (Beddard, 1904), and it will probably be found a general feature of the family *Gekkonidæ*

9 *Venæ Pulmonales*

Each *pulmonary vein* runs on the ventro-lateral border of the lung, receiving numerous minute factors from it It leaves the lung a little way back from the latter's anterior end, extends mesialwards, and meets its fellow to form the *common pulmonary vein* behind the heart The latter (Text-Fig 1, B) runs over the left side of the heart parallel and mesial to the left pulmonary artery, crosses the left part of the sinus venosus and opens into the left auricle

10 Summary

The author gives a detailed account of the heart and venous system of *Hemidactylus flaviviridis* The more important features discovered are as follows —

(1) The heart is situated considerably forwards in the pleuro-peritoneal cavity The sinus venosus is strongly fluted so as to accommodate the trachea which lies closely addressed to it There is no mesial constriction in the sinus venosus as described in *Uromastix hardwickii* The right auricle is devoid of the sac-like diverticulum The lumen of the ventricle is divisible into four regions passing insensibly into each other (*a*) the apical portion, subdivided by numerous trabeculæ, (*b*) the region in which the muscular ridge originates, (*c*) the region of the auricular apertures, and (*d*) the region of the aortic trunks There is, in addition to the muscular ridge, a supplementary ridge opposite to it in direction The openings of the aortic arches are situated at the free border of the muscular ridge

(2) The disposition of the aortic arches has been described

(3) The *vena trachealis* and *V jugularis communis* meet each other, and the combined trunk, thus formed, joins the *vena subclavia* to form the *anterior vena cava*

(4) The left tracheal vein is smaller than the right, and there are hardly any anastomoses between them—a condition presumably more primitive than in other lizards

(5) The *external jugular* vein is absent, so that the *internal jugular* drains the whole cephalic region

(6) The *venæ renales revehentes* arise intra-renally and are connected to each other by two anastomoses The right one is larger than the left and extends forwards, after receiving the blood of the left side by a transverse anastomosis, as the *posterior vena cava*

(7) The left *spermatic* vein opens into the left *vena renalis revehens*, while the right one opens into the *posterior vena cava*

(8) There are a number of minute *oviducal* veins opening into the *venæ renales revehentes*

(9) The *suprarenal portal system* is absent

(10) The point of bifurcation of the *caudal* vein to form the *venæ renales advehentes*, lies anterior to that in *Sphenodon* The two *venæ renales advehentes* are equi-sized

(11) A branch of the *vena renalis advehens* runs in the cleft between the anterior and posterior lobes of the kidney to join the *iliac* vein

(12) The *vena renalis anterior* is a branch of the *vena iliaca communis*, independent of the *vena anastomotica renalis*

(13) The *vena gastrica anterior* is a separate vein and has nothing to do with the *anterior abdominal vein*

(14) The *vena gastero-linealis* and *V intestinalis posterior* unite to form a common trunk

(15) There is a single æsophageal vein

(16) The *right and left pulmonary* veins unite to form the *common pulmonary* vein behind the heart,

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CYTOLOGICAL STUDIES IN INDIAN
PARASITIC PLANTS

II The Cytology of *Loranthus*

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IN a previous paper in this series (Kumar and Abraham, 1941 *a*), we described the cytology of *Striga* The present paper briefly deals with the cytology of *Loranthus longiflorus*, Desr

Loranthus species are hemi-parasites and the present material was collected locally from a mango tree Due to the presence of large quantity of mucilage and tannin, proper fixation of the buds was difficult The anthers in the immature condition are fused together by their margins to form a tube Excellent smear preparations were obtained by dissecting out the anther tube and laying it with the inner side in contact with the slide and smearing with a scalpel, applying a little extra pressure, and immediately fixing in Nawaschin's fluid or in La Cour 2 BE Staining was done by the usual iodine-gentian violet method

Mitosis

The somatic chromosomes were studied from sections of the young ovule There are eighteen chromosomes, two of which are satellited while the others have nearly median constriction (Fig 1)

Meiosis

Meiosis was studied entirely from smear preparations In smears stained with acetocarmine the spiral structure of chromosomes was evident from leptotene stage onwards But as the chromosomes are not large enough it was not possible to critically follow all the details of structure

At diplotene nine bivalents are clearly seen The nucleolus persists up to this stage, though only faintly stained, and one of the bivalents is usually attached to it

The chiasma frequency at diplotene is very low and most of the bivalents are held together by a single chiasma In acetocarmine preparations it was seen that in such cases the major spirals of the chromosomes are free

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from each other, suggesting that the mechanism of spiralisation is of the "balanced type" (Abraham, 1939) or "anorthospiral" (Kuwada and Nakamura, 1940)

Though the free arms of the bivalents are seen diverging from each other at the diplotene stage (Fig 2), by diakinesis they come together again and at metaphase (Fig 3) they are seen close together It is not possible to say whether this is a fixation effect or not, but it is significant that it was observed in a number of cases both in permanent smears as well as in temporary acetocarmine preparations

The second division is normal and nine chromosomes were counted at both metaphase plates in the same mother cell

Nucleolus

There are two nucleolar chromosomes seen in the somatic complement and at diplotene one bivalent is seen attached to the nucleolus An interesting feature seen from leptotene to pachytene is the presence of what appears like nucleolar buds As the chromosome threads are very fine and very distinctly stained, it was possible to study the relationship of these bodies to the chromosomes In Fig 4, a nucleolar bud is seen in contact with the nucleolus and two leptotene chromosomes attached to it In Fig 5 the nucleolar bud is connected to the nucleolus by two very fine
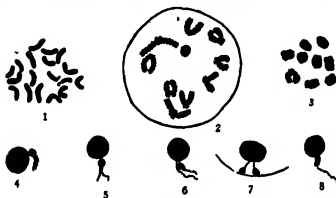
TEXT-FIGS 1–8

Fig 1 Somatic chromosomes from cell of ovule (2 *n* = 18) Note two satellited chromosomes.
Fig 2 Diplotene, showing nine bivalents, seven of which possess only one chiasma each
Fig 3 Metaphase showing nine bivalents Figs 4–8 Nucleolus and knob-like structure on nucleolar chromosomes (*vide* text) All figures are camera lucida drawings made from permanent preparations, Magnification of Fig 1 is ×3,000, while all the other figures are ×2,000

threads, which may be the continuation of the chromonemata In deeply stained preparations the small nucleolar bud is always seen in contact with the nucleolus, probably due to the masking of the gap due to retention of stain, as in Fig 8 In Figs 6 and 7, there are two nucleolar buds each attached to a chromosome These clearly indicate that what has been called a ' nucleolar bud ' for the sake of convenience of description may not actually be the result of budding of the nucleolus, but may be only an accumulation of nucleolar matter at the constriction between the satellite and the main body of the nucleolar chromosome Whether this is really so or only a knob of the type seen in *Euchlæna* chromosomes (Longley, 1937) could not be determined In *Sesamum orientale*, we found a secondary nucleolus originating from the primary nucleolus and persisting throughout both divisions of meiosis and ultimately getting enclosed in one or other of the four daughter cells (Kumar and Abraham, 1941 *b*) But the knob-like structures seen in the present case are most clearly visible from leptotene to pachytene and later they disappear, even before the main nucleolus disappears The origin of nucleoli and their possible role in nuclear and cell division have been discussed in detail elsewhere (Kumar and Abraham, 1941 *c*)

Summary

The cytology of *Loranthus longiflorus* is briefly described There are eighteen chromosomes in somatic cells and two of these are satellited Nine bivalents were seen at the first meiotic division The presence of a knob-like swelling on the nucleolar chromosomes near their attachment to the nucleolus is seen from leptotene to zygotene

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ON THE BIOLOGY OF RED SPIDER MITE
(*TETRANYCHUS TELARIUS* LINN.)
IN BALUCHISTAN

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Introduction

The Red Spider Mite (*Tetranychus telarius* Linn —Family Tetranychidæ, Acarina), which is widely spread all over the world, is fairly common in the hilly tracts of Baluchistan where it is one of the major pests of some of the deciduous fruit trees, vegetables, cultivated plants, etc In view of its importance, a detailed study of the biology of the mite has been made during the last three years and the results are recorded in this paper The writer feels indebted to Sri M C Cherian, Government Entomologist, Coimbatore, and Dr Khan A Rahman, Government Entomologist, Punjab, for their valuable criticism, to Mr A M Mustafa, Agricultural Officer in Baluchistan, for his able guidance, to the Imperial Institute of Entomology, London, for the identification of the species and to M Sabir Janjua, Entomological Fieldman, Quetta, for his help in the field and laboratory

Distribution

The Red Spider Mite is cosmopolitan, it is widely distributed between the tropical zones and North Germany It is also found all over North and South America, Hawaii Islands, South Africa, Australia and Palestine It is fairly common in Punjab and South India In Baluchistan, it is widely distributed in the hilly tracts, its attack being particularly severe in the districts of Quetta-Pishin and Loralai This is the first record of the pest from this province

Host Plants

The Red Spider Mite is markedly polyphagous, its food plants numbering over 300 different species in various parts of the world (Zacher, 1928) These include weeds, shade and forest trees, ornamental plants, garden and field crops Its attack is particularly severe on plants grown in green-houses in Europe and America McGregor (1917) has recorded 183 host plants of the mite in America where it is a serious pest of cotton, deciduous fruit trees, citrus varieties and a majority of vegetables. In

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Europe its host plants are weeds, shrubs, fruit trees, ornamental and green-house plants Sixty-three host plants of the pest have been registered in Palestine which include fruit trees, vegetables, fodder crops, ornamental plants, wind-break and forest trees (Klein, 1936) Its attack is, however, more severe on Citrus trees In South India, Cherian (1938) has recorded the mite on a variety of plants, the more important of which are ganja (*Canabis sativa*), castor (*Ricinus communis*), tomato, Cambodia cotton (*Gossypium hirsutum*), rose and jasmine (*Jasminum sambac*) In Punjab, it is a serious pest of a large majority of plants such as lady's finger (*Hibiscus esculentus*), "tinda" (*Citrullis vulgaris* var *fistulosus*), pulses such as "mothe" (*Phaseolus aconitifolius*), "mung" (*P mungo*), "mash" (*P radiatus*), "desi-sem" (*Canavalia ensiformis*), "ghiatori" (*Luffa ægyptica*) and sweet-potato (*Ipomœa batatas*) (Rahman and Sapra, 1940)

The following 32 host plants have so far been observed by the writer in Baluchistan Those that are severely attacked are almond (*Prunus amygdalus*), apple (*Pyrus malus*), apricot (*Prunus armeniaca*), carnation (*Dianthus caryophyllus*), celery (*Apium graveolens*), cherry (*Prunus cerasus*), chrysanthemum, geranium, grape-vine (*Vitis vinifera*), nectarine (*Prunus persica* var *Nucipersica*), peach (*Prunus persica*), pear (*Pyrus communis*), plum (*Prunus communis*), poplar (*Populus* sp), potato (*Solanum tuberosum*), rose (*Rosa muschata*), strawberry (*Fragaria* spp), tomato (*Solanum lycopersicum*), walnut (*Juglans regia*) and willow (*Salix* spp), while those that are less attacked are ash (*Fraxinus excelsia*), cabbage (*Brassica oleracea*), cucumber (*Cucumis sativus*), dahlias (*Dahlia* spp), egg-plant (*Solanum melongena*), lettuce (*Lactuca sativa*), melon (*Cucumis melo*), pea (*Pisum sativum*), turnip (*Brassica Rapa*), verbena (*Verbena* spp), violet (*Viola* spp) and water-melon (*Citrulus vulgaris*) The above-mentioned plants, however, represent only a part of the total number of host plants in this province and future research is sure to reveal further host plants and swell the list greatly

Injury

The mites feed upon the undersides of the leaves which they first cover with silken threads and then suck the sap by piercing the epidermis A small white spot develops around each feeding puncture which at first shows only on the underside of the leaf, later on the area becomes translucent as the spots coalesce When these punctures become numerous, the foliage loses its healthy green colour, turns pale and appears sickly The leaves so affected do not function normally and often turn rust-red before they crumple and dry up In severe infestations, the mites spin webs of such thickness over the foliage that the leaves practically cease to function The

buds may also be attacked, and even if they are not, they may still fail
to produce normal flowers if the leaves are badly infested Infestations,
when very severe, not only result in the plants being badly webbed but
also cause them to die

Developmental Stages and Seasonal History

The female after hatching from the egg passes through three quiescent
and three active stages, while the male goes only through two quiescent
and two active stages The stages in the life-history are as follows —

Female	*Male*
Egg	Egg
Larva	Larva
Quiescent Stage 1	Quiescent Stage 1
Protonymph	Protonymph
Quiescent Stage 2	Quiescent Stage 2
Deutonymph	Adult
Quiescent Stage 3	
Adult	

The Egg (Fig 1)—The egg is round, spherical in shape and when
newly laid is semi-transparent, globular and whitish It gradually turns to
a light straw colour and shortly before hatching becomes opaque and
pink On an average the egg measures 0 108 mm in diameter

The eggs are deposited singly on the underside of leaves amongst the
fine strands of webbing spun by the female They are hardly visible to
the naked eye The largest number of eggs deposited by a single female
was 72, and the largest number of eggs laid during a single day was 15
Eight, nine and eleven eggs a day are frequently laid during April and May
A single female on an average lays 37 eggs—ten females laying 370 eggs
During winter season the incubation period of the eggs was 6 days From
May to October the incubation period averaged 3 6 days One brood
deposited in July hatched in 2 days The average length of the incubation
period for the entire season was 4 9 days

The Larva (Fig 2)—When the larva is about to hatch, the shell of the
egg splits and the larva which is round and of the same size as egg, crawls
out of it The newly hatched larva is colourless except for the bright
carmine eye-spot and has only six legs It begins feeding immediately and
gradually turns light green in colour A small black spot appears at each
side of the body behind the eye-spot On an average the larva measures
0 15 mm in length and 0 12 mm in width
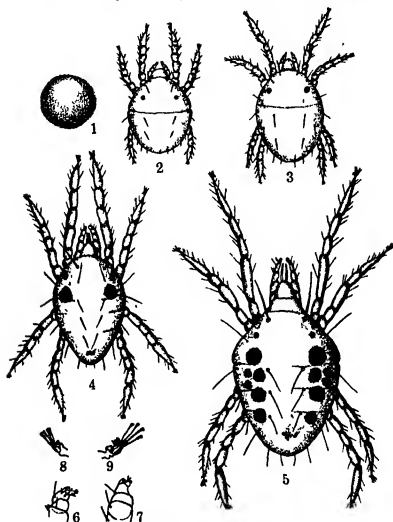
The Red Spider (*Tetranychus telarius* L)

Fig 1	Egg	×200	Fig 6	Palp of male
Fig 2	Larva	×200	Fig 7	Palp of female
Fig 3	Protonymph	×200	Fig 8	Claw of first leg of male
Fig 4	Adult male	×200	Fig 9	Claw of legs of female
Fig 5	Adult female,	×184		

Highly magnified

The average time required for the larva to attain maturity was 2 6 days for the whole year   The larvæ developed in 3–4 days from January to March, in 2–3 days from April to May and in 1–2 days from June to September

When the larva attains its growth, it enters a resting stage (Quiescent Stage 1)   The mouth parts are thrust into the tissue of the plant, the front two pairs of legs are extended parallel to each other and projected forwards while the hind pair of legs extend along the sides of the body   During the months of December, January and February this resting period will last for about one day but during the warmer months this resting period lasts only a few hours

Protonymph (Fig 3) —The larva undergoes a moult, when the larval skin, underneath which a new skin has been developed, breaks and discloses the protonymph   In addition to being larger, this differs from the larva in that it has four pairs of legs instead of three pairs   The dark spots at the sides of the body are slightly larger and the bristles on the body are longer   The abdomen becomes yellowish with brown spots on the sides, cephalothorax and legs are of the same colour but the tips of the legs are reddish   On an average the protonymph measures 0 22 mm in length and 0 15 mm in width

The nyumphs of this stage are somewhat more active than the larvæ but their habits are similar   They begin feeding soon after the moulting is completed   The average time required for the completion of this instar was 2 1 days   As with the larvæ, this instar lasted much longer during the colder months   It required 2–3 days from January to March, 1–1½ days from April to May, and about one day from June to September   They pass through a resting or premoulting period (Quiescent Stage 2) which lasts for an average of one day during the winter months and for only six hours during the more active period

Some protonymphs become much elongated and develop to male while the females remain more or less rounded in shape   The sexes are distinguishable from each other immediately on hatching from the egg by the structure of the palps and the claws of the first leg (Figs 6, 7, 8 and 9)

Deutonymph —It has been observed that the males do not pass through this stage but develop directly from the protonymphs   The females, however, pass through this additional stage which differs from the first in that the body becomes larger and longer   The abdomen becomes yellowish brown with black spots on the sides, cephalothorax and legs are of light yellow colour while the tips of the legs remain reddish   The average length of females of this stage is 0 31 mm, and width 0 19 mm.   The average

length of time required for this nymphal stage was 2 6 days, during cooler months about three days and in summer months about one day This instar also passes through a short resting period (Quiescent Stage 3) before the last nymphal skin is shed

Adults (Figs 4 and 5) —The adult male is easily distinguishable from the female by its attenuated shape The female is broader and longer than the male The average length of 20 females is 0 42 mm and breadth 0 25 mm , while the average length of an equal number of males is 0 25 mm and 0 13 mm wide The females vary considerably in colour, ranging from a light green colour to black Occasionally light yellow and red individuals are observed, but most of the individuals are green in colour with darker spots at each side of the body Cephalothorax and legs are yellowish while the tips of the legs are reddish The variations in colour in the male are very slight Most of the individuals are light green in colour with small black spots on the side of the body and a prominent red eye-spot After feeding, black areas appear rather far forward on each side of the dorsal surface of both sexes and a string of dark pellets makes their appearance posteriorly in the duct of the excretory organ In the female these dark globular products of digestion become diffused over the whole abdomen and give the mite a black appearance shortly prior to death

In several cases the males were observed assisting the females to shed the last nymphal skin and in these cases copulation took place immediately following the female's complete freedom from the nymphal skin After mating, the females begin feeding almost immediately and pass through a short pre-oviposition period which was on an average 1 5 days, in summer oviposition began after 24 hours, in spring and autumn the period was 2 days while in winter it went up to even 3 days Generally speaking, males and females possess the same longevity, which varies with the various seasons of the year In winter, the adults lived upto 25 days, in spring up to 20 days, in summer up to 10 days and in autumn up to 18 days The average egg-laying period extended through a period of 15 1 days The longest oviposition period for a single female was 25 days

In winter the mites are gregarious, often gathering in vast numbers in one place, where they spin a common web in which they constantly move about They are found chiefly in cracks in the soil and shelter under rocks, crevices in the bark of trees and shrubs and among trash and weeds All the stages are met during the winter season and on very cold days when the temperature goes far below the freezing point, they become inactive but even then sunlight will induce their multiplication and they resume their

ctivities It has been estimated that there are approximately 21 generations of the mite in a year in the Quetta Valley (5,500 ft.) Generations developing during the winter months required a much longer time (22-28 days) than those developing during the summer months (9-12 days)

The life-history may be summarized as follows —

Summarized Life-history Data of Red Spider Mite (Female)

Stage in life-history	Maximum (days)	Minimum (days)	Average (days)
Incubation period of eggs	6	2	4 9
Larval period	4	1	2 6
Quiescent Stage 1	1	4 hrs	12 hrs.
Protonymph Stage	1	1	1 9
Quiescent Stage 2	1	6 hrs.	14 hrs
Deutonymph Stage	3	1	2 1
Quiescent Stage 3	1	3 hrs	10 hrs
Life of adult	25	10	18 7
Pre-oviposition period	3	1	1 5
Number of eggs deposited per female	72	21	37

In some females parthenogenesis was observed and the unfertilized females were found laying eggs

Natural Enemies—At Quetta, *Adalia decempunctata* Linn (Family Coccinellidæ) and *Chrysopa* sp (Family Chrysopidæ) are the natural enemies of the Red Spider Mite, but these are not found in large numbers as to check the pest

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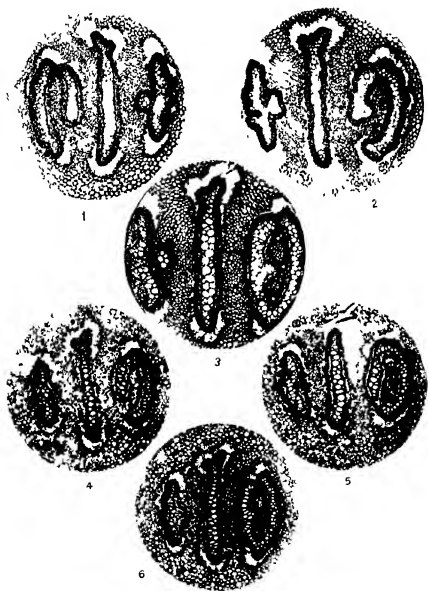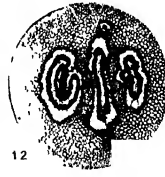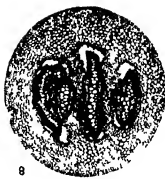
STAGES IN THE SPERMATOGENESIS OF
SIPHONOPS ANNULATUS MIKAN. AND *DERMOPHIS*
GREGORII BLGR. (AMPHIBIA : APODA)

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Received March 12, 1942

(Communicated by Prof A Subba Rau, D.sc , f r.m.s)

For some years past the author's interest has been aroused by the peculiarities in the gametogenesis of the members of the Apoda and has found expression in a number of papers on the various aspects of its study India is the home only of four genera of Apoda of which two are very rare. The more common genera (*Ichthyophis* and *Uræotyphlus*) which have been studied by the author have given indications of the importance of the study which has tempted him to continue his investigations and extend them to the other genera of Apoda The present paper is the result of this effort and marks an attempt to consolidate our available knowledge of the spermatogenesis of this interesting group In this the author has not been as successful as he would have wished to have been The material which forms the subject-matter of this paper is not Indian and evidently had not been treated for the study of the chromosomes The chromosomes, their number and behaviour,—have formed a very important part of my earlier studies on the spermatogenesis of *Ichthyophis* and *Uræotyphlus* and it is therefore with considerable regret that I state that I am unable to write on this aspect of the question. But feeling that it might be a very long time before fresh material may come my way, I have thought it fit to describe the general process of spermatogenesis in *Siphonops annulatus* and *Dermophis gregorii* and compare it with that in *Ichthyophis glutinosus* and *Uræotyphlus narayani*

B1

The specimen of *Siphonops annulatus* was fixed in corrosive sublimate acetic and later transferred into alcohol The fixation of *Dermophis gregorii* is not known Both of them had been lying in alcohol for a long time before they came into my possession but the preservation of the former is very much the better of the two It has given me all the information I wanted on the spermatogenesis of this species, except the chromosomes The preservation of *D gregorii* is not very satisfactory but some of the stages stand out clearly and have lent themselves for a correct and clear interpretation

I wish to thank Prof A Subba Rau for his kindness and encouragement throughout the course of this study and for his many helpful suggestions.

Structure of the testis —All the Apoda agree in the disposition of the testis. It occurs as a number of beaded structures strung together along the collecting duct as has already been observed in *Ichthyophis* and *Uræotyphlus* and as noticed again in *Dermophis* and *Siphonops* The number of testis lobes, however, is subject to variation, not only in the different species but also in the different individuals of the same species. I found only three lobes on each side in the specimen of *D gregorii* that I dissected. I cannot say if this is the normal number found in the species At any rate it represents the lowest number of testis lobes found by me in any Apodan example The size of the individual testis lobes is also subject to variation and the remarks I made about the significance of this variation in *Ichthyophis* and *Uræotyphlus* apply to the present genera also

The microscopic structure of the testis of the two genera under examination shows a close similarity with that of *Ichthyophis* and *Uræotyphlus* The external appearance of each testis lobe shows a number of rounded elevations which have been described by earlier workers (Spengel, 1876; Tonutti, 1931) as resembling a bunch of grapes Each of these elevations represents a locule and a number of such locules make up the testis In regard to the size of the locules the genera under examination differ a little, those of *Dermophis* being slightly the larger of the two

In the matter of the microscopic structure of the testis, the Apoda exhibit two distinctive characters. First, the disposition and size of the locules both show a very distinct and clear departure from either the urodelan plan on the one hand or the anuran plan on the other The locules are very large in the Apoda as compared with the Urodela or the Anura and are separated by clear thin septa (Fig 1). Each locule is filled with a fatty matrix in which are embedded cell groups in different stages of spermatogenesis. This matrix is very characteristic of the Apoda and has been found by me

in all the four genera examined and has also been found by Tonuttı (1931) in *Hypogeophıs* The presence of this matrix imparts an appearance to the testis which is different from the crowded nature of the cells in the testis of either the Anura or the Urodela In the latter groups, the comparatively small sized locules and the large number of cells (in different stages of spermatogenesis) give a packed appearance to the entire organ,
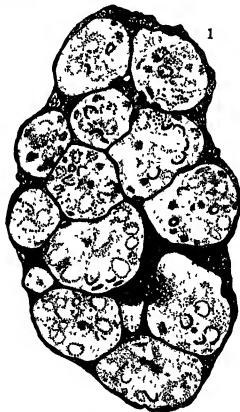
which is not seen in the testis of the Apoda at any time of the year or in any period of spermatogenesis Never at any time have I seen the locules of the testis packed fully with cells as in the other two groups of Amphibia. Even at the height of its activity much of the space in the locule is occupied

by the fatty matrix and it is only in this matrix that the germ cells lie embedded. Indeed, it would appear that this matrix is essential for the development of the cells themselves

The second point of interest is the uniformity of this testis structure throughout the group Apoda So far the structure of the testis of five genera is known, four studied by me and one (*Hypogeophis*) by Tonutti (1931) and in all these the peculiar and unique microscopic appearance of the testis described above, is seen In this respect, therefore, the Apoda is a close knit homogeneous group

To summarize the unique features of the testis of the Apoda consist in (*a*) the segmented nature of the organ which is resolved into a number of lobes strung along the collecting duct and which extends over a greater part of the length of the animal on either side of the alimentary canal, (*b*) the large size of the locules of the testis, these locules are not tubular as in the Urodela or the Anura but are more or less spherical and vary in number according to the size of the testis lobe, (*c*) the peculiar arrangement, in groups, of the sex cells in the locules Each locule exhibits a number of cell groups each of which is in a certain stage of spermatogenesis and such groups occur scattered and embedded in the fatty matrix that fills the locule Generally the cell groups in earlier stages of spermatogenesis occur near the periphery of the locule while those in later stages and those undergoing spermateleosis occur nearer the centre of the locule This arrangement is unique in the Amphibia, in the other two groups of which neither the fatty matrix nor the scattered arrangement of cell groups is found, and where cells in different stages of spermatogenesis are packed together in the available space found in the narrow tubules

The question may be asked if it is possible to account for this variation in the external and internal structure of the testis of the Apoda A partial answer may probably be found for the former The lobed nature of the testis may be a result of the elongation of the body of the animals of this group Even this is only a partial answer, for, while elongation of the body brings about, on the analogy of other animals, an elongation of the organs and also an asymmetrical development of the organs of the two sides, it does not ordinarily produce a segmentation of the organ It is probable that the reason for the lobing of the testis in the Apoda is a more fundamental and deep-seated one and must be looked for in the ancestral condition of the group. An answer to this cannot be provided at this stage

The origin and significance of the second structural feature of the testis is even more obscure The very large size of the testis locules as compared

with those of the other groups of Amphibia is as inexplicable as the development of the peculiar matrix filling the locules

The relation between the collecting duct and the locules has been described by me in *Ichthyophis* and *Ureotyphlus* where I have shown that the longitudinal collecting duct runs through the testis following the contour of the locules and giving off short side branches to the latter. The locules of *Dermophis gregorii* resemble those of *Ichthyophis* and *Ureotyphlus* in size while those of *Siphonops annulatus* appear to be much smaller comparatively. Spengel (1876) in his observations on the structure of the
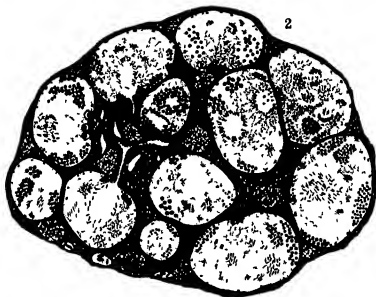
testis of Apoda indicates the relation between the testis locules and the collecting duct such that the latter runs in the centre of the testis with the locules arranged radially around it. In *Ichthyophis* and *Ureotyphlus* this regular relationship between the two could not be distinguished and it was observed that the collecting duct followed irregularly the interstices of the locules giving off smaller ducts to the locules. I find the same kind of arrangement in *Dermophis* also. In *Siphonops* on the other hand, a very slight trace of this central position of the duct and the peripheral position of the locules is seen (Fig.17). The main collecting duct appears to be central, with the locule.

arranged peripherally. But it must be mentioned that this arrangement i
not constant in *Siphonops* as seen in Fig 2.

Spermatogonia—My remarks regarding the origin of primary spermato-
gonia in *Ichthyophis* hold good for the two genera under examination.
I have pointed out how in *Ichthyophis* the cells lining the ducts of the testis
undergo transformation and so become germ cells Large numbers of these
cells were constantly found at the mouth of the duct in the locule in *Ichthyo-
phis* and *Uraeotyphlus* I believe, in the two genera under examination also
the duct mouth forms a constant source of primary spermatogonia in the
adult (Fig 3) Some of these cells at the mouth of the duct become large
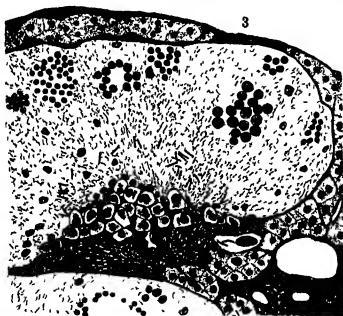
with conspicuous spherical and polymorphic nuclei while others invest
them and become the follicle cells From the close similarity which the
sections of the testis of *Siphonops* and *Dermophis* present to the sections of
the testis of *Ichthyophis* and *Uraeotyphlus*, I have reasons to believe that the
origin and behaviour of the primary spermatogonia is very similar.
Arising in this position the primary spermatogonia migrate along the wall

of the locule taking up positions along it (Fig 19) where they start to divide and grow and pass through meiosis.

The size of the primary spermatogonium is subject to great variation, according to its age and activity   In *Siphonops* it varies from 25 to 40 microns while in *Dermophis* the variation is between 16 and 30 microns

The problem of polymorphism of the nuclei of primary spermatogonia has been dealt with by me in sufficient detail in *Ichthyophis* (1936) and in *Uraeotyphlus* (1939) and I need not dwell on it here at any great length
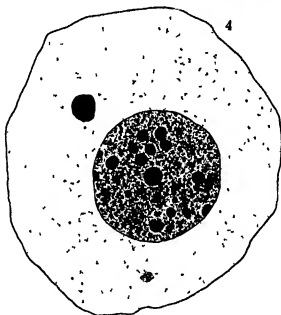
My conclusions regarding polymorphism are amply borne out by my observations on *Siphonops* and *Dermophis*   The spherical form of the nucleus denotes an earlier condition and the polymorphism (Figs. 6 and 20), which is never very pronounced and which is similar to that encountered in other Apoda indicates a condition of particularly heightened metabolic activity The nucleus however, always reverts to its spherical form just before division (Figs. 7, 8 and 22)   The number of nucleoli is subject to great variation and from a condition where there are one or two nucleoli (Fig. 5)

to one where there are several (Figs. 4 and 21), all gradations occur. As in *Ichthyophis* and *Uræotyphlus* the staining reactions of the nucleus of the primary spermatogonium also vary according to its spherical or polymorphic condition, being deeper in the former and fainter in the latter

Of the cytoplasmic bodies I am able to speak only of the centrosome, for this, along with the contained centrioles, is the only object that is at all preserved in the material Even the centrioles are not always preserved well enough to be seen clearly This is especially so in regard to *Dermophis* where the fixation is not as good as in *Siphonops* In this latter material, however, the centrioles are quite clear and occupy the centre of the archoplasmic area which bears the same relationships with the nucleus and cell in general as in *Ichthyophis* and *Uræotyphlus* (Fig 21) These relationships leave me in no doubt as to the topography of the different cytoplasmic inclusions of the primary spermatogonium, which, had they been well fixed and preserved, would have revealed the same arrangement as in *Ichthyophis* and *Uræotyphlus*

A word about the amitotic divisions of the nucleus of the primary spermatogonium In my paper on *Ichthyophis* (1936) I discussed this matter fully and subscribed to the view of Wilson (1928) that amitosis here, as elsewhere, means nothing more than a fragmentation of the nucleus with an attendant increase in the nuclear surface and that in no case could a division significance be attached to it In *Ichthyophis* I did find, though extremely rarely, a few isolated instances of binucleate spermatogonia In *Siphonops* during my examination of the very limited material at my disposal I found a single primary spermatogonium with two nuclei (Fig 23) I was unable to determine the nature and condition of the cytosome but I feel that it is unnecessary to deviate from the conclusions drawn in case of *Ichthyophis*, that amitosis here, as in *Ichthyophis*, has no division significance and means nothing more than a temporary change involving an increase of nuclear surface

The onset of division brings about a conversion of the polymorphic nucleus into a regular spherical condition and so far as I can see, the changes that occur in the nucleus and cytoplasm are similar to what I have reported already in *Ichthyophis* and *Uræotyphlus* The nucleus shows blocks of chromatin connected by filamentar processes and these are the forerunners of the definitive chromosomes A stage of the kind is shown in Fig 7 The difference between the nucleus in a state of rest and a stage just prior to division can be seen on comparing Figs 21 and 22 In the former, the nucleus appears granular, the granules filling the cavity of the nucleus more or less evenly, while in the latter, the nucleus appears like a vesicle the chromatin having
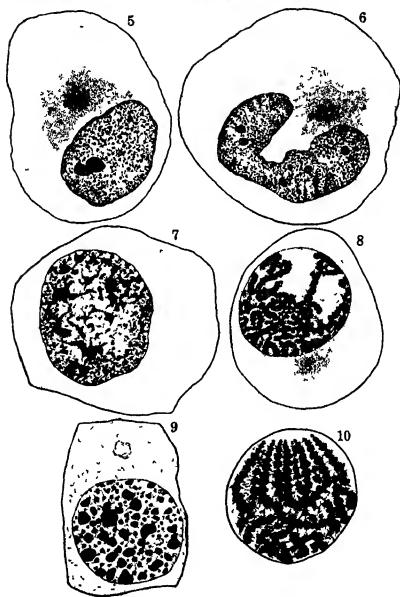
become aggregated into a few coarse masses leaving conspicuous clear spaces in between them This difference was also observed in the two genera studied earlier

The primary spermatogonium divides by mitosis and the products of division remain together just beneath the locule septum. The number of divisions a primary spermatogonium passes through before meiosis starts has been a matter of interest to me and I have determined that in *Ichthyophis* the number of divisions is eight, while in *Uraeotyphlus* it is more irregular, the primary spermatogonium passing, sometimes through six divisions and sometimes through seven.

In the present two genera also I tried to count the number of divisions the primary spermatogonium passed through before meiosis set in and so far as the material at my disposal enabled me, and based on the countings of the metaphase plates of the first meiotic division, I found that in *Siphonops* the number of divisions was seven and in *Dermophis* it was slightly irregular, being either six or seven From an examination of the conditions obtaining in the four genera of Apoda I have so far studied, I am in a position to conclude that the number of divisions a primary spermatogonium in the Apoda passes through before meiosis, though subject to some variation, is six, seven or eight So far it is only in *Ichthyophis* I have noticed evidences of eight divisions

Spermatocytes—After the completion of the divisions by mitosis, the cells, which are now the primary spermatocytes, enter on the meiotic phase. At first, as in *Ichthyophis* and *Uraeotyphlus*, these cells are arranged along the periphery of the locule in two rows (Fig 24) and form a compact mass But as meiosis proceeds, the cells leave their peripheral position and migrate inwards into the locule, the individual cells of the same group occurring together but the cell groups themselves being separated from one another and lying in the matrix filling the locule (Figs 1 and 2)

I have observed both in *Ichthyophis* and *Uraeotyphlus* a stage of rest intercalated between the last division of the spermatogonia and the prophase of meiosis of the spermatocytes In this condition the nucleus shows a large number of blocks of chromatin But there is no attendant diminution of basophily of the nucleus as has been observed in a number of Amphibia, notably in *Rana* (Witchi, 1924) and in *Bufo* (Saez and others, 1936). In *Siphonops*, where this stage could be observed with great clearness, it was even more pronounced than either in *Ichthyophis* or in *Uraeotyphlus* (Figs. 9 and 25)

The first stages of meiosis appear to be similar to those described in the two Indian genera The leptotene bouquet is built up as in *Ichthyophis* and *Uræotyphlus* The polar orientation of the leptotene threads is evident even from the start and as in the above two genera, the threads begin to be
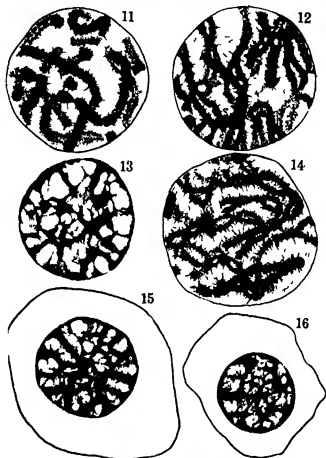
formed at the pole, the rest of the nucleus displaying unthreaded granules The pachytene stage is clear and conspicuous with its thicker threads (Figs. 10 and 26) and forms, as in *Ichthyophis*, by far the most stable stage of meiosis and is one of longest duration Gradually the polar orientation of the

threads of the pachytene nucleus is lost and soon the threads lie anyhow inside the nuclear cavity spanning it (Figs 11 and 17) Meanwhile splits and spaces are appearing inside each thick bivalent chromosome and the duality of each of these is clear and evident (Figs 12 and 28)

Associated with the diplotene stage has been described in the Apoda a conspicuous stage of diffusion of chromatin where the individuality of the earlier diplotene chromosomes is temporarily lost in an indistinguishable network characteristic of all the Apoda The ' diffuse ' stage has been described in a variety of plants and animals and while it appears as a more normal phenomenon in the development of the oocyte in animals, its occurrence in spermatogenesis is relatively rare but more interesting Chickering (1928) has given a detailed account of this stage in the spermatogenesis of Belostomatidæ (Hemiptera) and his observations coincide with mine in the Apoda (1937, 1939) Chickering traces the development of diffusion in *Lethocerus* step by step in which the first step is marked by a separation of the two univalents at intervals, their transverse movement and a fine branching My Fig 15 of *Ichthyophis* (1937) and his 59 of *Lethocerus* are strikingly similar. And Chickering concludes " a coarse reticulum is formed by a continuation of this process",— a statement which is very similar to my description of the process in *Ichthyophis* The extension of my observations of this stage to *Ureotyphlus* and now to *Siphonops* and *Dermophis* substantiate my conclusions arrived at in case of *Ichthyophis* and I have reasons to believe that the 'diffuse' stage is a universal character of the Apoda, following the diplotene stage and arising in the same manner as that described by me in *Ichthyophis* A nucleus in the 'diffuse' stage is shown in Fig 13

The ' diffuse ' stage is of fairly long duration at the end of which the chromosome bivalents emerge gradually from the network characteristic of the diffuse condition The final stages of this condensation show the bivalents still long and thin and bearing a large number of transverse filamentar processes (Figs 14 and 29) The chiasmata can be made out clearly now and it is seen that in some of the larger bivalents the chiasmata are quite large in number. This character of the large number of chiasmata in the early stages of diakinesis was noticed in *Ichthyophis* and also in *Ureotyphlus* where 6 to 7 chiasmata were observed In these two animals it was noticed that the number of chiasmata were gradually reduced till the largest bivalent in either form did not have more than four chiasmata in the final condition. Unfortunately I am not, on account of the unsuitability of fixation of the material, able to describe the history of the chiasmata or trace the fate of the chromosomes into metaphase.

The division stages succeed each other rapidly, and, intercalated between the first and second meiotic divisions there is a definite stage of rest (Figs. 15 and 30) of fairly long duration as observed by me in *Ichthyophis* and *Uræotyphlus* The spermatids are formed after the second division (Fig. 16)

The following measurements give the diameter of the nucleus in the different stages of spermatogenesis in the two genera under examination

Stage	*Siphonops annulatus* microns	*Dermophis gregorii* microns
Primary spermatocyte at rest	9 to 10	5 to 6
Pachytene stage	11 5 to 12	10 to 11
Diffuse stage	12 5 to 13 5	11 to 12
Diakinesis	15 to 16 5	13 to 13 5
Secondary spermatocyte	10 to 10 5	6 to 8
Spermatid	6 to 7	4 to 5

Summary

The testis structure of the two genera described here shows that it conforms to the plan outlined for *Ichthyophis glutinosus* and *Uræotyphlus narayani* except that in *Dermophis gregorii* very few testis lobes were seen The testis locules are smaller in *Siphonops annulatus* when compared with those of the other three genera The locules are filled with a matrix which in *Ichthyophis* and *Uræotyphlus* were determined as containing fat. In this matrix are embedded the germ cells in groups in different stages of spermatogenesis. The primary spermatogonia are found at the mouth of the duct in the locule and are believed to have arisen, as in *Ichthyophis*, from the cells lining the duct epithelium Their nuclei may be spherical or polymorphic, the latter condition indicating a high degree of metabolic activity Just before division, however, the nucleus resumes its spherical or oval contour After a number of divisions, varying between six and eight, the cells,—now primary spermatocytes,—embark on the meiotic phase after a brief period of rest The leptotene and pachytene stages follow, after which, the nucleus is marked by a 'diffuse' condition in which the chromosome bivalents lose their identity temporarily and the whole nucleus presents the appearance of a resting stage When the bivalents emerge from this network, their chiasmata are clear and in the larger bivalents they are quite large in number though they are probably reduced later as in *Ichthyophis* and *Uræotyphlus*. After a brief interkinesis the second division occurs giving rise to the spermatids

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EXPLANATION OF FIGURES

1   Longitudinal section of a testis lobe of *Dermophis gregorii* The large locules are separated by thin septa and contain cell elements in various stages of spermatogenesis Groups of interstitial cells are also seen   × 50.

2   Transverse section of a testis lobe of *Siphonops annulatus* illustrating the general plan of structure.   × 70

3   A part of the longitudinal section of a testis lobe of *Dermophis gregorii* showing a group of primary spermatogonia at the mouth of the duct.   × 266

4   *Siphonops annulatus*. A primary spermatogonium with a spherical nucleus and several nucleoli Two nucleoli extruded into the cytoplasm are also seen   × 3100.

5   *Dermophis gregorii*. A primary spermatogonium with a slightly polymorphic nucleus   × 3100.

6   *Siphonops annulatus* A primary spermatogonium with a polymorphic nucleus.   × 2266.

7   *Siphonops annulatus* A primary spermatogonium preparing for division   × 2266

8   *Siphonops annulatus*. A primary spermatogonium in prophase   × 2266

9   *S. annulatus*. A primary spermatocyte with nucleus in a stage of rest   × 3100.

10   *S. annulatus* A pachytene nucleus.   × 3100.

11   *S annulatus* Beginning of the diplotene stage. The loss of polar orientation of the bivalents is seen.   × 3100.

12   *S. annulatus*. Early diplotene. Splits have appeared at intervals along the bivalents   × 3100.
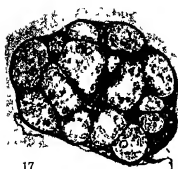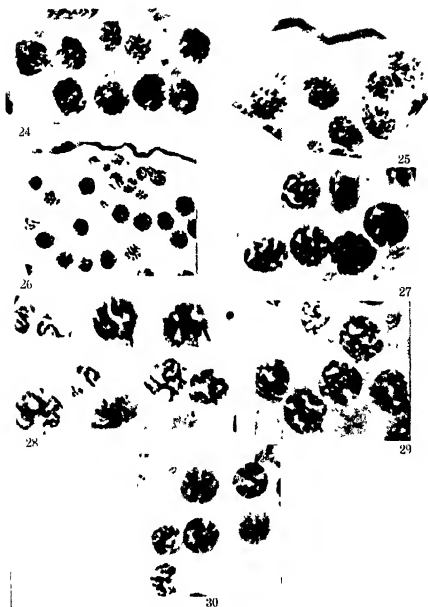
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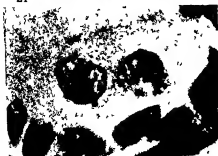
13 *S. annulatus* Nucleus of the primary spermatocyte in the 'diffuse' condition. × 3100.

14 *S. annulatus* Later stage The bivalents have become recondensed and the chiasmata are visible × 3100

15 *S. annulatus* Secondary spermatocyte with the nucleus in interkinesis × 3100

16 *S. annulatus* Spermatid × 3100

17 *S. annulatus* Photomicrograph of a transverse section of a testis lobe. × 35

18 *S. annulatus* Photomicrograph of a testis locule showing the opening of the duct into the locule × 200

19. Photomicrograph of the periphery of a locule of the testis of *S. annulatus* showing three primary spermatogonia close to the septum Their investing follicle cells are also seen. The nucleus of one of the spermatogonia (the middle cell) is preparing for division. The contrast between this cell and those on either side is sharp and clear (see text). × 700

20 A primary spermatogonium of *S. annulatus* showing its polymorphic nucleus × 1300

21 *S. annulatus* A primary spermatogonium with a polymorphic nucleus and many nucleoli. The centrosome with the centrioles is also clear × 1400

22 A primary spermatogonium of *S. annulatus* preparing to divide The nucleus is vesicular with the chromatin aggregated into blocks × 1400

23 A binucleate primary spermatogonium of *S. annulatus* × 1400.

24 Early spermatocytes of *S. annulatus* lying in two rows beneath the septum of the locule. × 1400

25 *S. annulatus* Primary spermatocytes at rest × 1400

26 A group of pachytene nuclei of *S. annulatus* × 400

27 *S. annulatus* Beginning of the diplotene stage. The polar orientation of the bivalents is lost. × 1400

28 *S. annulatus* Later stage. Splits in the bivalents are clearly seen × 1400

29 *S. annulatus* Nuclei showing the emergence of the bivalents from out of the diffuse stage. × 1400

30 *S. annulatus* Interkinesis × 1400

ORIGIN OF INTRALOCULAR OOCYTES IN MALE APODA

By B. R. Seshachar

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THREE years ago I reported the occurrence of three oocytes in the testis of *Uræotyphlus narayani* (Seshachar, 1939). The ova which were all intra-locular were fairly well advanced in development and were characterised by their large size, the germinal vesicle condition of their nuclei and the large number of nucleoli. That was the first time oocytes were found in the testis of any Apodan example and it was concluded that they were formed as transformations of primary spermatogonia. This conclusion is now amply borne out by the discovery in *Ichthyophis glutinosus* of an oocell in the testis which bears all the characters of a transforming spermatogonium and I am now able to corroborate Witschi (1934) that intratubular ova in the testis are derived from a transformation of primitive gonia.

During a recent examination of fresh material of *Ichthyophis glutinosus* I noticed in a locule of the testis a cell which bore all the marks of an oocyte, but which had not advanced in development so much as the oocytes described by me in *Uræotyphlus*. This material happened to have been fixed in Kolatschev's fluid and the sections had been mounted unstained. The oocell was large and extended over four sections each of seven microns thickness. Its appearance in two of them has been figured here. In one, the nucleus is seen, in the other, what is obviously the archoplasmic region is figured.

The oocyte is larger than a normal primary spermatogonium though much smaller than the ova described in the testis of *Uræotyphlus narayani*. It has a definite shape which is maintained by the development of a conspicuous envelope,—two features which at once led me, along with the large size, to distinguish the cell from the primary spermatogonium. Moreover, while the primary spermatogonium is always found either at the mouth of the duct or at the periphery of the locule, the oocyte projected into the cavity of the locule, a position which is never ordinarily occupied by the primary spermatogonium. The nucleus is slightly polymorphic and has a single nucleolus. The size of the nucleus is similar to that of the primary spermatogonium but the cytoplasm definitely showed evidences of increase in volume. The Golgi elements which are seen in the section figured in B, are in the form of numerous discrete crescentic bodies of various sizes arranged in a ring around a more or less clear space, the archoplasmic area. In the

G Golgi body, M Mitochondria, N Nucleus, NL Nucleolus
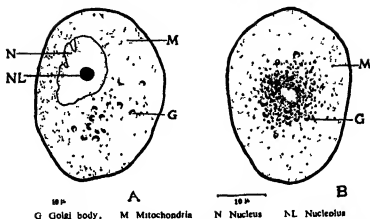
primary spermatogonium, the Golgi apparatus is in the form of a compact body investing the sphere (Seshachar, 1936) This difference between the normal primary spermatogonium and the oocyte just described is very clear and striking The intensity of osmication of the structures in the two cells also differs In the oocyte the Golgi bodies were only slightly blackened while in a primary spermatogonium close by, the Golgi apparatus was deeply black The mitochondria however, remained scattered in the cytoplasm

From the above description of the oocyte it will be clear that it represents one of the stages through which a primary spermatogonium passes in its transformation into an oocyte The small polymorphic nucleus and the single nucleolus (there are usually many in the fully developed oocyte) are characters of the primary spermatogonium while the large size and definite shape of the cell and its thick envelope remind us of the oocyte It is evident that the first changes that take place during ' oviform degeneration' affect the cytoplasm The increase in its quantity as well as the development of a definite envelope are only two of these changes The Golgi apparatus which occurs as a compact body in the primary spermatogonium has become broken up here into discrete elements The mitochondria appear to be the only structures which are unaffected, they occur scattered in the cytoplasm as they do in many cases in the primary spermatogonium

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ANALYSIS OF RASPURI AND BADAMI VARIETIES OF MANGO (*MANGIFERA INDICA*) GROWN IN MYSORE*

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Two varieties of the mango fruit, Raspuri and Badami, are largely grown and eaten in Mysore in the earlier part of the mango season which lasts for about three months from March to June The badami variety is preferred to the raspuri and it is the popular belief that the former does not bring about any digestive trouble In order to know the difference in composition that might exist between the two varieties we undertook a chemical analysis of the edible portion of the fruit. While doing so, instead of taking only the fruits sold in the market for analysis we thought it would be interesting to investigate the variations of several factors like pH, mineral contents, sugars, etc., during its development Ranganathan and co-workers (1937) have determined moisture, mineral matter and carbohydrates in the green and ripe mangoes grown in Coimbatore and Salem Shri Ranjan and Jha (1940) have estimated the mono- and di-saccharides in the edible portion The former authors have calculated the value for carbohydrates by difference and the latter have used Pavy's solution for the determination of sugars It is unnecessary to discuss here the relative merits of the methods used for sugar determinations However, we have used Lane and Eynon's copper titration method for reducing sugars (1923) and Hanes for finding out the ratio of fructose to glucose (1929)

Experimental

Collection of samples —Four healthy trees, two badami and two raspuri, were selected for experiment in the Mysore Municipal Farm Samples were collected between 7-30 and 8 A M every week for three months till the fruits were plucked for storage The fruits were weighed immediately and the edible part cut, making use of stainless steel knife The skin and the stone were discarded An average sample was made use of for the analysis As for the ripe fruit, samples were bought in the local market.

Moisture —15-20 gm were dried at 50-51° C to a constant weight in an electric oven thermostatically controlled

Total mineral matter —10-15 gm were heated slowly in a porcelain crucible till carbonisation took place and then ashed and weighed to constant weight A qualitative analysis of the ash showed the presence of carbonate, phosphate, chloride, iron, manganese, sodium and potassium

pH of the expressed juice - 100 - 200 gm were mechanically pressed in a fruit juice extractor and pH determined electrometrically using a quinhydron electrode The values were checked by the colorimetric method

Titratable acidity of the extract with alcohol —The extract prepared as given below under sugar estimation was titrated against 0 1 N NaOH using phenolphthalein as the indicator

Sugars —100 gm were extracted with 200 c c of neutral 95% alcohol at laboratory temperature in a percolator for 12 hours, the liquid was separated from the solid and kept aside The residue was subjected to hot extraction with 125 c c of 80% alcohol in a Soxhlet apparatus for 16 hours continuously The two extracts were mixed the alcohol from the mixture was distilled off under reduced pressure (8-10 cm) at 35 to 38° C The residue was made up to 200 c c with water The solution was treated as given by Archbold and Widdowson (1931), and the reducing sugars estimated by Lane and Eynon's method (1923) using their sugar table As the de-leaded solution was free from colouring matter, it was not boiled with charcoal The ratio of fructose to glucose was determined by using alkaline ferricyanide (Hanes, 1929, Widdowson, 1931) Sucrose was estimated by hydrolysing with 10% citric acid, neutralising with NaOH and estimating the total sugars by Lane and Eynon s copper titration method Starch was not detected

The following table gives the results of the analysis

B2a

Badami (Municipal Farm)

No of the sample	Date 1940	No of fruits taken	Average wt. of fruit in gm	pH of expressed juice	Moisture	Ash	Titratable acidity in c.c. 0.1 N NaOH	Fructose	Glucose	Sucrose	Remarks
					for 100 gm						
1	30 3	14	28 5	2 20	87 7	0 2810	474			0 31	
2	6 4	14	42 0	5 51	88 8	2986	572	0 88	0 44	15	
3	13 4	14	69 0	4 95	88 4	2208	566	90	44	11	Rain
4	20 4	14	89 7	5 26	88 4	2137	638	72	24	02	"
5	27 4	14	165 0	5 64	89 6	2012	566	70	58	22	"
6	4 5	14	186 0	4 02	89 7	2025	654	55	42	24	
7	11 5	14	207 5	5 24	85 5	1794	602	74	48	22	
8	18 5	14	250 0	5 34	83 5	2787	512	66	60	46	Rain
9	25 5	14	244 3	3 20	81 0	2693	172	83		41	
10	1 6	12	241 0	5 48	81 3	2874	468	70	93	58	
11	8 6	12	267 6	5 84	79 8	3349	264	70		53	
12	15 6	14	226 5	5 60	75 1						

Market Sample

No of the sample	Date 1940	No of fruits taken	Average wt. of fruit in gm	pH of expressed juice	Moisture	Ash	Titratable acidity in c.c. 0.1 N NaOH	Fructose	Glucose	Sucrose	Remarks
1	11 6	6	286 0	6 7	81 1	3354	44	2 64	2 22	4 98	
2	19 6	6	240 0	5 2	78 8	4322	60	2 14	1 86	3 72	
3	26 6	6	286 0	6 3	81 4	4582	80	2 35	1 60	5 17	
4	6	6	241 0	6 3	80 7	4366	64	2 23	1 77	4 01	
Average for market sample				6 1	80 5	4156	62	2 34	1 86	4 47	

Raspuri (Municipal Farm)

No of the sample	Date 1940	No of fruits taken	Average wt. of fruit in gm	pH of expressed juice	Moisture	Ash	Titratable acidity in c.c. 0.1 N NaOH	Fructose	Glucose	Sucrose	Remarks
1	30 3	14	18 2	3 99	87 6	2500	412			0 19	
2	6 4	14	50 0	3 27	88 0	2824	592	0 88	0 40	13	
3	13 4	14	69 0	5 16	88 5	2324	606	1 00	46	29	
4	20 4	14	113 0	5 43	88 6	2298	634	72	29	29	Rain
5	27 4	14	187 2	5 57	89 0	2307	585	66	36	43	"
6	4 5	14	226 3	4 71	89 4	2668	588	68	23	19	"
7	11 5	14	226 3	4 81	87 0	2302	522	67	34	34	
8	18 5	14	282 3	5 08	84 0	2661	512	64	55	49	Rain
9	25 5	14	276 5	5 18	82 4	2458	432	60	68	68	
10	1 6	14	292 6	5 29	80 1	2907	388	90	66	40	
11	8 6	12	263 0	5 90	78 7	2846	324	1 22	1 00	1 28	

Market Sample

No of the sample	Date 1940	No of fruits taken	Average wt. of fruit in gm	pH of expressed juice	Moisture	Ash	Titratable acidity in c.c. 0.1 N NaOH	Fructose	Glucose	Sucrose	Remarks
1	11 6	6	233 3	7 51	81 7	3071	68	2 60	2 00	6 76	
2	19 6	6	364 2	5 87	77 6	3690	88	2 48	1 47	4 53	
3	26 6	6	345 0	6 03	81 3	3384	84	1 65	1 53	5 60	
4	6	6	261 0	6 21	84 0		72	2 31	1 98	4 74	
Average for market sample				6 4	81 1	3382	78	2 26	1 74	5 41	

N.B.—The blanks in the above table indicate quantities not estimated

Comparative results of the analysis of the Mango fruit

Place	Variety	pH of expressed juice	Moisture	Ash	Titratable Acidity in c.c. 0.1N NaOH	Mono-sac-charides	Di-saccha-rides	Total carbo-hydrate	Authors
Coimbatore Salem United Provinces	Green Ripe 30—50 days old 60—110 days old	4.04 to 4.9	90.03 86.14	0.37 0.32		1.32 to 6.3 2.6 3.6 to 2	1.10 to 9 3.1 to 2	8.84 11.69	Rangaswamishan and others Shri Ranjan and Jha
Mysore	Badami green, 1 to 15 weeks old Badami ripe Raspuri green, 1 to 15 weeks old Raspuri ripe	2.30 to 5.84 6.1 3.06 to 6.4	75.1 to 89.7 80.5 78.7 to 89.0 81.1	0.1794 to 0.3349 0.4156 0.2916 to 0.2846 0.3382	6.54 to 2.64 6.2 6.34 to 3.24 .78	0.96 to 2.06 4.20 4.91 to 2.22 4.00	0.13 to 1.53 4.47 1.13 to 1.28 5.41		Authors

Summary

Analysis of the edible portion of the badami and raspuri varieties of the mango fruit grown in Mysore has been made during the different stage of its development, with a view to finding out the change in moisture content, ash, pH, titratable acidity, the reducing sugars and sucrose

The values obtained are tabulated to show the differences between the two varieties at different stages. It is particularly of interest to note that the badami variety contains a greater percentage of minerals and less of the sugars than the raspuri

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Hanes	*Ibid* 1929, **23**, 99
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ENZYMIC PROTEOLYSIS*

Part V   The Liberation of Cystine

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INVESTIGATIONS on the time course of the liberation of cystine and other amino-acids from proteins during enzymic digestion were carried out by Abderhalden and Reinbold (1905)   In these experiments pancreatin was allowed to act upon edestin, portions of the digest were removed at definite intervals and dialysed   The non-dialysable residue gave negative reactions for tyrosine after 24 hours, for tryptophane after 4 days and for cystine (" Schwefelblei probe ") after 8 days, indicating that these three amino-acids were present entirely in the free condition at the end of the respective periods noted   Recently the rate of liberation of cystine has been studied more quantitatively with the aid of two widely used methods, the Folin and Marenzi method, based upon the reduction of Folin's uric acid reagent by thiol compounds, and the Sullivan reaction using β-naphthaquinone sulphonate   Employing these colorimetric procedures Jones and Gersdorff (1933) found that during peptic digestion of casein the Sullivan reaction remained negative throughout while the Folin and Marenzi reagent gave high colour values which rose to a peak in the early stages of digestion and then fell to a constant value   In parallel experiments in which hydrolysis was effected by means of 20% HCl the Folin and Marenzi method showed similar abnormal values which were, in the early stages of hydrolysis, much higher than the total cystine content of casein   Jones and Gersdorff came to the conclusion that pepsin does not split free cystine from casein and that the high Folin and Marenzi colour values obtained with peptic digests are produced by some compound or compounds other than cystine   In their opinion two factors, a stable and an unstable type of chromogenic compound, were involved, the possibility was also suggested that substances such as furfural, pyruvic acid, levulinic acid etc , might be responsible for colour formation   With egg albumin using the same reagent for the estimation

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of cystine Calvery et al (1936) obtained high chromogenic values but in contrast to Jones and Gersdorff appear to be of opinion that these values represent in fact cystine set free by pepsin

In tryptic digestion there is evidence to show that cystine is rapidly liberated Pollard and Chibnall (1934) using Prunty's (1933) modification of the Sullivan reaction found that about 72 and 52% respectively of the cystine in rye grass and cocksfoot proteins were present in the free condition after 72 hours' digestion with trypsin These experiments also gave indication of the decomposition of cystine during prolonged digestion In experiments on the tryptic digestion of casein carried out by Jones and Gersdorff (1939) 80% of the cystine was liberated in 24 hours, but a large part of this was destroyed on account of the alkalinity of the medium (pH 8–9)   At pH 6 6–6 8 about 40% of the cystine as determined by the Sullivan method was in the free condition in 24 hours and almost complete liberation took place in 120 hours   In these experiments also the Folin and Marenzi method gave "high and erratic values"

In the experiments now described a study has been made of the liberation of cystine from eight proteins during the action of pepsin and trypsin in succession   As in the experiments of Jones and Gersdorff (1933) none of the eight proteins studied gave a positive Sullivan reaction during peptic digestion, but with the uric acid reagent all gave high colour values   The value in the case of casein (Fig I) reached a maximum at 161% of the total cystine of casein in the first hour of digestion and then fell gradually to a constant value of about 104%   Egg albumin (Fig II) showed a similar peak
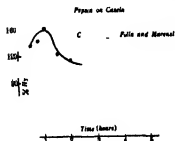
FIG I
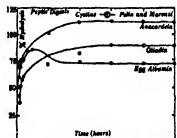
FIG II

value of 86 5% at the end of 12 hours   The remaining six proteins (Figs II and III) showed no such maxima, the Folin and Marenzi colour values increasing gradually in the course of digestion and finally remaining at a constant high level   In the case of anacardein this constant value was higher

again than the cystine content of the protein In every case it was found that colour production with the Folin and Marenzi reagent could be completely inhibited by H CHO and HgCl₂, as inhibition by these reagents has been shown by Shinohara (1936) and Lugg (1932) to be specific for thiol groups
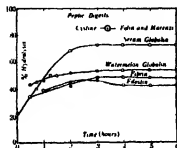
Fig III
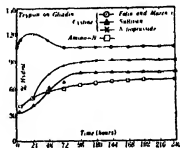
Fig IV

it can be concluded that the chromogenic substances formed during peptic digestion are cystine complexes, most probably large polypeptides, capable of producing a higher intensity of colour than cystine itself In the case of casein and egg albumin which show pronounced maxima in the hydrolysis curves it has to be assumed that complexes formed in the earlier stages of digestion are later split up into substances with less colorigenic power

In tryptic digestion there were similar discrepancies between the values obtained by the two methods Folin and Marenzi method gave with all the proteins, with the exception of edestin, values higher than the total cystine content, gliadin (Fig IV), watermelon globulin (Fig V) and ana-cardein (Fig VI) showing maxima in the early phases of digestion similar
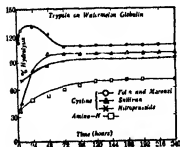
Fig V
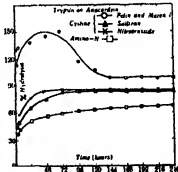
Fig. VI

to those shown by casein and egg albumin in peptic digestion   As colour production in these digests also could be completely inhibited by H CHO and $HgCl_2$ it is to be inferred that the chromogenic substances are cystine peptides

Sullivan values also showed maxima in two cases, viz , casein and fibrin However, while the shape of the curves with Folin and Marenzi values is to be explained on the assumption of the formation and subsequent break-down of highly chromogenic polypeptides the rise and fall of the Sullivan values observed with these two proteins is to be ascribed to destruction of free cystine In view of the evidence for the destruction of cystine produced by Jones and Gersdorff (1939) at the end of the digestion period aliquots of the digests were hydrolysed completely with acid and cystine determinations carried out by the Sullivan method   With most proteins it was found that there was considerable destruction, the value obtained in the digests being much lower than on an equivalent amount of the intact protein   The extent of destruction varied from protein to protein being highest with casein, amounting to almost half, egg albumin and watermelon globulin showed no destruction while the other proteins occupied intermediate positions   In addition to the Folin and Marenzi and Sullivan methods, the nitroprusside reaction (Krishnaswamy, 1942) was also made use of in studying the progress or tryptic digestion   The values for disulphide groups thus obtained were usually intermediate between the values obtained by the other two methods but it does not appear that any special meaning can be attached to these data

From a comparison of the rates of peptide splitting (increase in amino nitrogen) and cystine liberation (as determined by the Sullivan method) it will be obvious that in some digests cystine splitting takes place much more rapidly than peptide hydrolysis so that there is justification for the view that this amino-acid occupies an " exposed position " in these proteins   The data regarding the extent of peptide hydrolysis at the stage when cystine liberation had reached the maximum are summarised in Table I Making allowance for the amount of cystine destroyed during digestion it will be seen that practically all the cystine in anacardein, watermelon globulin and edestin are set free in the early stages of digestion (48–72 hours) when peptide hydrolysis has proceeded to only about 50%   From gliadin 95% of the cystine is set free in 96 hours when the extent of protein hydro-lysis is 58%   With the animal proteins, fibrin, egg albumin and serum globulin, however, there is no such marked discrepancy between the rates of cystine and amino nitrogen liberation, cystine present in the free condi-tion ranging from 74–80% when peptide hydrolysis is approximately 70%

The significance of these observations with reference to the position of cystine in the protein molecule requires further elucidation

TABLE I

Liberation of Cystine and Amino-N

Protein	Time hr	Peptide Hydrolysis %	Free cystine % (corrected for destruction)
Edestin	72	59 3	97 2
Gliadin	96	58 7	95 4
Anacardein	72	56 9	100 9
Watermelon globulin	48	53 2	99 1
Casein	24	49 5	90 2
Fibrin	96	68 6	78 8
1 gg albumin	120	65 2	74 4
Serum globulin	168	71 3	80 7

Experimental

Materials—Casein, edestin, gliadin and fibrin were prepared according to standard methods The serum globulin used was the euglobulin described in Plimmer (1933) Anacardein was prepared according to Damodaran and Sivaswamy (1936) and the watermelon globulin by the method of Krishnan and Krishnaswamy (1939) B D H preparations of egg albumin, pepsin and trypsin were used

Enzymic Digestion—The following procedure based upon that employed in previous work from this laboratory (*cf* Damodaran and Ananthanarayanan, 1938) was uniformly employed with all proteins studied To 20 g protein dissolved in about 950 ml of N/20 HCl was added, after adjustment of pH to 1 8, 1 g pepsin in 50 ml of N/20 HCl and the solution made up to 1 l At the end of 7 days 450 ml of the digest was brought to pH 8 3 with NaOH, 0 5g trypsin dissolved in a small amount of water added and the solution made up to 500 ml

For control, 1g pepsin was dissolved in 950 ml of N/20 HCl, the pH adjusted to 1 8 and the solution made up to 1 l After 7 days 450 ml were brought to pH 8 3 and made up to 500 ml after the addition of 0 5 g trypsin dissolved in water The values for amino-N and cystine in the digests were in all cases corrected for the values in the control experiments

Methods of Analysis—Amino-N was determined by formol titration according to Sorensen (Abderhalden, 1923) 5 ml aliquots of digests were withdrawn at intervals, neutralized to pH 6 8 and the resulting solution

after treatment with 10 ml of neutralised formalin titrated with standard N/10 NaOH

Cystine was determined by the Folin and Marenzi (1929) method as modified by Tompsett (1931), the Sullivan method (Sullivan and Hess, 1937) and by means of nitroprusside (Krishnaswamy, 1942) Aliquots measuring 2 to 5 ml were removed according to the concentration of cystine present Further details were exactly as described in the original papers In casein digests with low cystine content determinations had to be made with added standard In the accompanying tables, A, B and C represent Folin and Marenzi, Sullivan and Nitroprusside values respectively

For calculation of the extent of peptide hydrolysis amino nitrogen determined according to Sorensen was compared to the amino nitrogen on complete hydrolysis, this value being calculated from N-distribution values given in Plimmer (1917) For calculation of % cystine splitting total cystine in each protein was determined experimentally after acid hydrolysis For ascertaining the extent of the destruction of cystine during digestion the digests were completely hydrolysed with acid and cystine determinations made in these hydrolysates

Total Cystine in the Proteins—0 5 to 3 g of protein (to contain about 10 mg cystine) was hydrolysed with 20 parts of 20% HCl for 8 hours, the hydrolysate repeatedly distilled *in vacuo* to expel the acid, decolorised with 0 5 g kaolin and made up to 50 ml 5ml aliquots were used for the determination of cystine by the three colorimetric methods already mentioned From the values given in Table II it will be seen that with completely hydrolysed proteins, with the exception of casein, the three methods give fairly concordant values

TABLE II

Cystine Content of Proteins

Protein	Cystine %		
	A	B	C
Casein	0 42	0 28	0 29
Edestin	1 44	1 41	1 48
Gliadin	2 52	2 39	2 61
Anacardein	1 45	1 38	1 34
Watermelon globulin	1 19	1 19	1 19
Egg albumin	2 69	2 15	2 69
Serum globulin	2 41	2 05	2 53
Fibrin	2 02	1 84	2 28
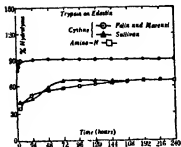
Fig. VII
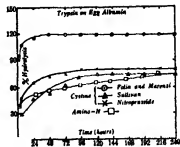
Fig VIII
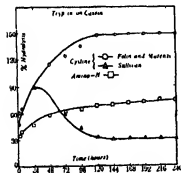
Fig IX
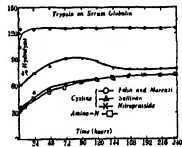
Fig X
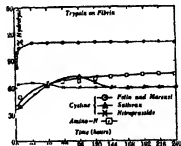
Fig XI

Destruction of Cystine during Tryptic Digestion.—10 ml of the digest in each case was treated with 17 ml of conc HCl and refluxed for 8 hours The acid was removed by repeated distillation *in vacuo*, the solution decolorised with kaolin and made up to 25 ml 5 ml aliquots were taken for

Sullivan determination, standard cystine being added in the case of aliquots with low cystine content   The results are given in Table III

TABLE III

Destruction of Cystine

Protein	Cystine % in Protein	Cystine % in Protein after digestion	% Destruction
Edestin	1 41	0 97	31 3
Gliadin	2 39	1 96	18 2
Anacardein	1 38	1 02	26 7
Watermelon globulin	1 19	1 19	0
Casein	0 28	0 15	45 8
Fibrin	1 84	1 73	5 9
Egg albumin	2 15	2 15	0
Serum globulin	2 05	1 89	7 9

PROTOCOLS

TABLE IV

Pepsin and Trypsin on casein

Time hr	Hydrolysis %				
	Pepsin		Trypsin		
	Peptide	Cystine A	Peptide	Cystine	
				A	B
0 5		135 7			
0·75		142 9			
1	5 9	161 1			
1 5		127 5			
2	6 5	114 3		47 1	
3	7 2	105 4	35 6	60 7	
4	7 9	104 3			
5	8 5	103 9			
6			38 6	65	60 4
24	9 8	"	49 5	90	90 2
48	11 9	"	57 4	114	60
72	13 9	"	60 9	123 2	58 2
96	15 6	"	63 8	133 9	43 6
120	16 9	"	66 8	146 1	32 9
144	17 3	"	69 0		30 9
168	17 3	103 9	70 9	"	"
192			72 7	"	"
216			74 4	"	"
240			75 6		
264			76 0	146 1	30 9
288			76 0	146 1	30 9

TABLE V

Pepsin and Trypsin on Edestin

Time hr	Hydrolysis %				
	Pepsin		Trypsin		
	Peptide	Cystine A	Peptide	Cystine A	B
1	2 1	39 2		78 0	
2	3 6	44 5		81 6	
3	7 2	45 8		86 5	
4		42 2	14 0		
6	8 4	42 0	37 3	90 0	42 4
24	11 7		51 4		46 8
48	13 9		56 3		59 0
72	14 8		59 3		66 2
96	15 5	"	61 4	"	"
120	16 0	"	62 9	"	"
144	16 2	"	64 1	"	"
168	16 2	42 0	65 1	"	"
192			66 1		
216			66 8		
240			67 5		
264					
288			67 5	90 0	66 2

TABLE VI

Pepsin and Trypsin on Gliadin

Time hr	Hydrolysis %					
	Pepsin		Trypsin			
	Peptide	Cystine A	Peptide	Cystine A	B	C
1	1 3	35 6		105 9		
2	3 3	42 9		107 9		
3	4 9	51 9	33 9	110 9	33 5	
4	5 9	56 9				
5	6 3	60 3				
6			39 0	114 2		
24	10 9		48 9	120 0	41	50 2
48	11 8	73 2	55 1	112 9	60 7	78 3
72	12 8	82 4	58 7	105 0	67 3	84 1
96	13 9	90 4	58 7		77 4	88 3
120	14 2		62 7			90 4
144			65 4			
168	14 2	90 4	67 3			
192			68 0			
216			68 7			
240						
264						
288			68 7	105 0	77 4	90 4

TABLE VII

Pepsin and Trypsin on Anacardein

Time hr	Pepsin		Trypsin			
	Peptide	Cystine A	Peptide	Cystine A	Cystine B	Cystine C
0 5		63 5		112 7		
½	4 5	70 6		130		
1	6 4	73 7			42 6	
2	7 4	77 3				
4	8 3	79 4	35 6			
5	9 0	85 8				
6	10 9		41 2	133 3	47 6	57 2
24	12 9	105	50 6	137 6	65 4	75 9
48	13 7	112 9	54 5	144 7	74 4	83 0
72	14 5	"	56 9	150	82 5	84 4
96	15 0	"	60 2	117 2	"	85 1
120	15 5	"	62 0	107 1	"	"
144			63 1	97 9	"	"
148	15 5	112 9	64 1	"	"	"
192			65 4	"	"	"
216			66 3	"	"	"
240			67 0	"	"	"
264				"	"	"
288			67 0	107 1	82 5	85 1

TABLE VIII

Pepsin and Trypsin on Watermelon Globulin

Time hr	Pepsin		Trypsin			
	Peptide	Cystine A	Peptide	Cystine A	Cystine B	Cystine C
0 5		43 0				
0 75		45 0				
1	1 9	46 1				
1 5		50 2		110 9		
2	3 3	51 3		117 6		
3	3 5	52 9	35 2	122 7	36 6	
4	7 2	"				
5	8 7	"				
6						
24	11 5	"	39 5	126 7	45 4	69 5
48	12 6	"	47 6	131 1	81 2	84 0
72	13 5	"	52 2	121 9	99 1	85 7
96	14 2	"	59 4	108 4	"	87 4
120	15 0	"	63 5		"	93 0
144	15 4	"	65 8		"	"
168	15 4	52 9	67 3		"	"
192			68 3		"	"
216			69 0		"	"
240			69 3		"	"
264			69 8		"	"
288			69 8	108 4	99 1	93 0

TABLE IX
Pepsin and Trypsin on Egg Albumin

Time hr	Pepsin Peptide	Pepsin Cystine A	Trypsin Peptide	Trypsin Cystine A	Trypsin Cystine B	Trypsin Cystine C
0.5	1.3	41.4				
1		42.6				
1.5		54.0				
2	2.7	61.4		93.0		38.5
3	3.8	70.0	39.5	94.2		
4	4.8	75.6				
5	4.7	77.2				
6	7.2	78.4	41.7	107.2	28.7	46.5
8	8.1	84.7				
12	10.2	86.5		113.3	45.7	65.2
24	11.5	75.6	41.6	115.3		
48	13.5	74.6	45.7	119.1	52.3	70.0
72	15.2	73.5	59.0	"	61.6	74.4
96	16.7		62.0	"	70.2	80.0
120	16.7		65.2		74.4	
144	19.4		68.1		"	
168	19.4	73.5	71.2		"	
192			73.3		"	
216			74.3		"	
240			76.3		"	
264			76.8		"	
288			76.8	119.1	74.4	80.0

TABLE X
Pepsin and Trypsin on Serum globulin

Time hr	Pepsin Peptide	Pepsin Cystine A	Trypsin Peptide	Trypsin Cystine A	Trypsin Cystine B	Trypsin Cystine C
0.75	1.4	22.1				
1		40.2				
1.25		43.9		103.9		
2	3.4	50.3	34.4	120.5	35.9	62.4
3	6.8	69.2		145.3		
4	8.2	72.2				
6		"	37.5			
24	11.1	"	47.8		52.7	74.1
48	13.2	"	54.1		54.6	81.5
72	14	"	59.1		62.2	91.2
96	15.4	"	64.3		64.1	91.2
120	15.8	"	68.2		66.5	88.3
144	16.0	"	70.2		68.4	79.0
168	16.0	72.2	71.3		72.7	
192			72.3		"	
216			73.5		"	
240			74.6		"	
288			75.3	145.3	72.7	75.0

TABLE XI

Pepsin and Trypsin on Fibrin

Time hr	Pepsin		Trypsin				
				Cystine			
	Peptide	Cystine A	Peptide	A	B	C	
0 5		34 2		83 2			
1	3 5	39 2		94 6			
2	7 5	41 9	40 6	100 2		64 1	
3	8 2	44 1		105 4			
4	9 3	"		110 3			
5	10 4	"			39 0	64 7	
6		"	46 1	"	53 8	65 3	
24	13 5	"	53 4	"	62 0	65 9	
48	15 5	"	60 5		63 1	60 3	
72	17 1	"	65 5		72 8	"	
96	18 5	"	68 6		65 8	"	
120	19 2	"	70 2		60 3	"	
144	19 9	"	71 7			"	
168	20 3	44 1	73 3		"	"	
192			74 5		"	"	
216			75 1				
240			75 8				
288			75 8	110 3	60 3	60 3	

Summary

A study has been made of the rate of liberation of cystine and amino nitrogen from eight proteins during successive digestion by pepsin and trypsin

Assuming the specificity of the Sullivan reaction it can be concluded that no free cystine is present in peptic digests

The same reaction shows that cystine is rapidly set free during the action of trypsin From the vegetable proteins (edestin, gliadin, anacardein and watermelon globulin) cystine is liberated much more rapidly than other amino-acids, practically the whole of the cystine in the protein being present in the free condition at a very early stage of proteolysis Such complete liberation does not take place with the animal proteins (serum globulin, egg albumin and fibrin) , with these, rate of cystine splitting approximates more closely to peptide hydrolysis In the course of tryptic digestion at alkaline pH destruction of cystine takes place, the extent of the destruction varying from protein to protein

The Folin and Marenzi reagent gives high colour values with both peptic and tryptic digests These values have no relation to the free cystine

present, being frequently in great excess of the total cystine content of the protein This colour production is due to cystine complexes and not to non-nitrogenous substances as has been previously suggested

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